Title: Increasing the dose density of adjuvant chemotherapy by shortening intervals between courses or by sequential drug administration significantly reduces both disease recurrence and breast cancer mortality: An EBCTCG meta-analysis of 21,000 women in 16 randomised trials

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Body: Background: Much contemporary adjuvant chemotherapy uses conventional 3-weekly scheduling. Yet, cytokinetic modelling suggests that increasing the dose density of cytotoxic therapy by shortening the intervals between courses, or by using sequential rather than concurrent treatment schedules may enhance efficacy.¹ At least 15 randomised trials have directly tested this hypothesis and this meta-analysis by the Early Breast Cancer Trialists’ Collaborative Group (EBCTCG) brings together the worldwide evidence to clarify the balance of risks and benefits of dose-dense chemotherapy.

Methods: Individual patient data were provided for 98% (21,537/21,944) of women randomised in relevant trials: 7 randomised trials (10,004 women, 2240 breast cancer recurrences, 1481 breast cancer deaths) that compared 2-weekly dose-dense chemotherapy versus the same chemotherapy given 3-weekly, and 9 trials (11,533 women, 2773 breast cancer recurrences, 1711 breast cancer deaths) that compared sequential with concurrent anthracycline and taxane-based chemotherapy. Primary outcomes were time to recurrence and breast cancer mortality.

Results: Highly significant reductions in disease recurrence [rate ratio (RR)=0.83 (95%CI 0.76-0.91), p=0.00004] were seen with 2-weekly compared with 3-weekly chemotherapy, and 10 year breast cancer mortality was 3.0% lower [16.7% vs 19.7%: RR=0.85 (95% CI 0.76-0.95), p=0.003]. Overall survival was also improved [RR=0.86 (95% CI 0.78–0.95), p=0.003]. Similarly, for sequential versus concurrent taxane plus anthracycline chemotherapy the rate ratio for disease recurrence was 0.86 (95% CI 0.79-0.93, p=0.0001), 10-year breast-cancer mortality was 2.3% lower [19.2% vs 21.5%: RR=0.87 (95% CI 0.79-0.96), p=0.005], and overall survival was improved [RR=0.85 (0·78–0·94), p=0.0008]. The proportional reductions in recurrence with dose-dense chemotherapy were similar and highly significant (both p<0.002) in ER-positive and in ER-negative disease, and did not differ significantly by any other patient or tumour characteristics, including age, HER2 status, nodal status, tumour size, or grade. Increasing dose density did not have any material adverse effect on non-breast-cancer mortality, which was similar with 2-weekly and with 3-weekly chemotherapy [RR=0·93 (95% CI 0·74–1·17), p=0.6] and was if anything lower with sequential than with concurrent chemotherapy [RR=0·73 (95% CI 0·55–0·97), p=0.03]. Trial publications also indicate that, with haematopoietic growth factor support, dose-dense chemotherapy does not substantially increase toxicity.

Conclusion: Increasing the dose density of adjuvant chemotherapy is safe and results in fewer disease recurrences and fewer deaths from breast cancer.

Reference:
Title: NSABP B-47 (NRG oncology): Phase III randomized trial comparing adjuvant chemotherapy with adriamycin (A) and cyclophosphamide (C) → weekly paclitaxel (WP), or docetaxel (T) and C with or without a year of trastuzumab (H) in women with node-positive or high-risk node-negative invasive breast cancer (IBC) expressing HER2 staining intensity of IHC 1+ or 2+ with negative FISH (HER2-Low IBC)

Body: Background: Adjuvant trastuzumab (H) reduces cancer recurrence and improves survival in patients (pts) with HER2-amplified or overexpressing (IHC 3+ staining intensity) IBC. Two of the landmark trials that demonstrated the efficacy of H-based eligibility on HER2 testing performed at local site laboratories were found to contain a cohort of pts without amplification or IHC overexpression on tissue submitted for central testing. These HER2-low cohorts appeared to benefit from the addition of H, and efforts at external HER2 testing validation and laboratory explorations did not negate these findings. NSABP B-47 was performed to determine if these findings would be confirmed in a large prospective randomized trial. The primary aim was to determine whether the addition of H to chemotherapy (CT) regimens of AC→WP or TC (choice per investigator discretion) would improve invasive disease-free survival (IDFS).

Methods: From 2/8/2011 to 2/10/2015, 3270 women were enrolled with 1630 pts randomly assigned to Arm 1 [TC: docetaxel 75mg/m2, cyclophosphamide 600 mg/m2 every 3 weeks x 6 cycles; or AC→WP: doxorubicin 60 mg/m2 and cyclophosphamide 600 mg/m2 every 2 or 3 weeks x 4 cycles followed by paclitaxel 80 mg/m2 every week x 12], and 1640 pts to Arm 2 [same CT regimens + 12 months of H]. Pts were stratified by IHC score (1+ vs 2+), number of positive nodes (0-3, 4-9, ≥10), hormone receptor status (ER or PgR positive vs both negative), and CT (TC vs AC→WP). Overall 58.5% were ≥50 years, 57% had tumors with IHC 1+, 17.3% were ER- and PgR-, 19.9% were node negative, and 27.4% had ≥4 positive nodes. TC was the intended CT regimen for 44.2%.

Results: As of 7/31/2017, the median follow-up time was 46.1 months. We observed 264 IDFS events, which triggered the definitive analysis for the primary endpoint. The addition of H to CT showed a 5-year IDFS of 89.6% compared to 89.2% for CT alone (HR 0.98; 95%CI 0.77-1.26; P=0.90). The findings did not differ by level of HER2 IHC expression, level of lymph node involvement, or hormone receptor status. 5-year point estimates for RFI were 92.0% for CT+H compared to 92.2% for CT alone (HR 0.995; 95%CI 0.75-1.32; P=0.97). 5-year estimates for DRFI were 92.7% for CT+H and 93.5% for CT alone (HR 1.10; 95%CI 0.81-1.49; P=0.55). The addition of H did not change OS significantly with 5-year point estimates of 94.8% in CT+H vs 96.2% in CT alone (HR 1.33; 95%CI 0.91-1.94; P=0.14). 4.3% of women in the CT arm experienced Grade 4 or 5 toxicities compared to 4.3% in the CT+H arm.

Conclusion: The addition of H to CT did not demonstrate a reduction in IDFS, RFI, or DRFI in women with non-overexpressing but IHC measurable HER2 IBC. This prospective study did not confirm the retrospective findings in NSABP B-31 or N9831. The threshold of HER2 expression or genetic amplification for H benefit remains unchanged.

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**Title:** Peri-operative aromatase inhibitor treatment in determining or predicting longterm outcome in early breast cancer – The POETIC* Trial (CRUK/07/015)

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**Body: Background**

Experimental evidence (Fisher et al, 1989) & a small clinical trial (IMPACT) respectively suggested peri-operative endocrine therapy (ET) may improve long-term disease-related outcome in patients undergoing primary surgery for ER positive (ER+) breast cancer (BC) & that tumor Ki67 levels after 2 weeks of peri-operative aromatase inhibitor (POAI) therapy might offer an effective way of predicting outcome & the need for additional adjuvant treatment. POETIC (*Peri-Operative Endocrine Therapy - Individualising Care) is a phase III randomized controlled trial designed to test these hypotheses & provide data to determine whether 2 week Ki67 improves prediction beyond that by baseline Ki67 of the group who have a higher risk of relapse in the first years after diagnosis in spite of best current standard of care.

**Patients & methods**

Postmenopausal patients with ER+ BC were randomised 2:1 to either, POAI (centre choice: letrozole 2.5mg or anastrozole 1mg daily) for 14 days prior to & 14 days following surgery or no POAI (Control). Randomization was stratified by treating center; adjuvant treatment was per UK routine practice. Tissue samples were collected at baseline & surgery (FFPE) for blinded Ki67 testing. Primary endpoint was Time to Recurrence (TTR: time from randomization to loco-regional or distant recurrence or BC death). A secondary endpoint was Ki67 at baseline & after 2 weeks of AI.

**Results**

Between 2008 & 2014, 4480 patients (2976 AI, 1504 Control) were randomized from 130 UK centers. Median age was 67 (IQR 62-75), 18% had grade 3 tumors, 39% were node positive and 61% had tumor size>2cm. For adjuvant ET 314 patients (7.2%) received tamoxifen (Tam), 3695 (84.6%) an AI, 251 (5.7%) Tam changing to AI and 109 (2.5%) changing from AI to Tam. On 8 August 2017, median follow-up was 60.7 months (IQR 49.5 to 72.2).

408/4480 (9.1%) patients have had a TTR event; 263 (8.8%) allocated to POAI compared to 145 (9.6%) controls: HR=0.91 (95%CI: 0.74, 1.12) Log-rank p=0.37. Adjusted HR=0.91 (95%CI: 0.74, 1.11).

The relationship of Ki67 (baseline & after 2 weeks) with TTR in both the POAI & control groups will be presented for the overall ER+ population & HER2 defined sub-groups.

**Discussion**

There was no significant evidence that four weeks of POAI improved TTR compared with no POAI. POETIC will provide definitive evidence on the role of 2 week POAI-treated Ki67 to inform future practice & trials in terms of the potential to identify a group of patients for whom current standard of care appears insufficient in the few years post diagnosis.
Title: Copy number aberration analysis to predict response to neoadjuvant anti-HER2 therapy: Results from the NeoALTTO phase III trial

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Body: Background
In the NeoALTTO trial, the combination of trastuzumab and lapatinib for the neoadjuvant treatment of HER2 positive early breast cancer patients (pts) nearly doubled the rate of pathological complete response (pCR) compared with either anti-HER2 treatment alone. In the same trial, pCR was shown to be a surrogate for long-term outcome. Expression of ERBB2, ESR1 and immune signatures were the main drivers of pCR (Fumagalli et al. JAMA Oncol 2016). The main aim of the current study was to investigate the relevance of copy number aberrations (CNAs) for pCR and event-free survival (EFS).

Methods
For 271 out of the 455 pts enrolled in the study with sufficient tumour content, DNA was hybridized on CytoScan HD arrays. The \( \log_{2} \) ratio intensities and B allele frequencies were segmented jointly using the multitrack segmentation algorithm. Integer level estimates of total copy number and major allele were obtained using GAP. Recurrent CNAs were found with GISTIC2. The genome instability index (GII) was defined as the median absolute deviation of the normalized copy number. Tumor infiltrating lymphocytes (TILs) and gene expression were obtained as described previously. The correlations between different parameters were assessed using Spearman correlations (\( \rho \)). The Mann-Whitney test was used to relate binary and numerical features. EFS analysis was performed using the Cox proportional hazard model.

Results
CNAs estimates were obtained for 185 out of 271 pts. CNA distribution was similar to the ones observed in HER2+ pts from the METABRIC and the TCGA datasets. There were 64% of diploid pts, 26% of triploid and 9% of tetraploid or more. Aneuploidy level was not associated with pCR or EFS. Of interest, there were many significant differences in CNA profiles between ER+ and ER- pts. Those differences mirror those observed between ER+ and ER- among HER2- pts in TCGA (\( \rho=56\% \)) and METABRIC (\( p=56\% \)). ERBB2 amplification was predictive of high pCR (\( p=0.0007 \)), albeit less so than ERBB2 expression, and ceased to be significant correcting for ERBB2 expression. The pCR rate increased with the GII (\( p=0.03 \)), independently of ERBB2 amplification. The effect was stronger in ER+ patients (\( p=0.01 \)). GISTIC analysis revealed 159 recurrent CNA regions. Amplification of 2 regions on 6q23-24 was significantly associated with higher pCR (\( p=0.00005 \) and \( p=0.00087 \), FDR=0.006 and 0.05). The most significant segment of 6q23-24 contained 39 genes, some whose expression level also predicted pCR (e.g. MAP3K5, \( p=10^{-4} \)). Gene ontology analysis of the genes correlated with this segment highlighted the category 'response to interferon-alpha' (\( p=4.3x10^{-7} \)). No amplified region or gene was found to be predictive of EFS, after multiple testing correction.

Conclusions
The amplification of ERBB2 was shown to be predictive of pCR, however its expression was more predictive. High genomic instability was associated with higher rate of pCR in ER+ subgroup. Of interest, a novel amplified region, involving chromosome 6 was shown to be predictive of pCR, which may warrant further investigation.
Title: Tandem duplicator phenotypes define 50% of triple negative breast cancers

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Body: Background. We recently discovered a unique chromotype, the Tandem Duplicator Phenotype (TDP), characterized by hundreds of somatic tandem duplications (TDs) scattered throughout the genome of a large percentage of triple negative breast cancers (TNBCs). Importantly, we observed that the TDP associates with a better response to cisplatin therapy in vitro and in vivo, suggesting that it is a tractable and quantitative biomarker of response to platinum-based therapy. Here, we expand on our initial findings by analyzing Whole-Genome (WG) sequences of over 2,700 tumors.

Methods. TD coordinates from WG sequences relative to 2717 tumors were assembled from over 30 independent studies representing several cancer types, including 254 TNBCs. WG sequencing of mouse breast tumors was carried out using standard Illumina protocols. The number, distribution and span-size of somatic TDs from a training set of 992 tumors were used to develop a TDP classifier that identifies highly recurrent but clearly distinct TDP profiles. The TDP classifier was then applied to the remaining tumor sequences. WG mutation and copy number datasets were investigated to identify the genetic drivers associated with each TDP profile, and the genomic consequences of different TDPs were evaluated through identification of genomic hotspots for gene duplication and transection.

Results. We describe six different TDPs featuring distinct TD span size distributions, with peaks at 10Kb (group 1), 300Kb (group 2) and 3Mb (group 3), or different combinations of these (mix12, mix13 and mix23). More than half of all TNBC display a TDP. Of these, 55% classify as group 1, 14% as group 2 and 15% as group mix12. Whereas all TDP groups show a higher TP53 mutation rate compared to non-TDP tumors, each TDP profile is characterized by specific additional gene perturbations, with loss of BRCA1 occurring in groups 1, mix12 and mix13; CCNE1 amplification in group 2; and CDK12 mutations in group mix23. We show that different TDPs are subject to the perturbation of specific oncogenic networks resulting from the duplication of oncogenes by larger TDs (>300Kb) or the disruption of tumor suppressors via double transections by shorter TDs (10Kb). Indeed, tumor suppressor genes such as PTEN, RB1 and MLL3 are frequently disrupted by TDs in TNBC TDP group 1 tumors, whereas TNBC TDP group 2 tumors commonly feature duplication of oncogenes such as MYC and MALAT1. Finally, through WG analyses of 18 mouse models (GEMMs) of breast cancer, we provide the first mechanistic evidence of the driving role of conjoint loss of TP53 and BRCA1, but not of BRCA2, in inducing the TDP group 1 profile.

Conclusions. Our study shows a definitive genetic induction of one specific form of TDP (group 1) characterized by 10kb TD span. Different TDP profiles are characterized by alternative somatic genetic origins but always couple with disruptive TP53 mutations. The consequences of the massive TD formation in TDP TNBCs suggest a systems strategy to tumor induction involving heterogeneous combinations of oncogenes and tumor suppressors. That these TDP forms, accounting for ~50% of TNBC, are associated with significant sensitivity to cisplatin suggest that this chromotype may identify TNBC patients who would benefit from upfront platinum-based chemotherapy.
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Title: Extended adjuvant bisphosphonate treatment over five years in early breast cancer does not improve disease-free and overall survival compared to two years of treatment: Phase III data from the SUCCESS A study

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Body: Background:
A recent meta-analysis has reported benefits of adjuvant bisphosphonate treatment in early breast cancer patients with improved breast cancer survival and reduced rate of breast cancer recurrences in the bone, especially in postmenopausal patients. However, a comparison between bisphosphonate treatments duration is lacking. Therefore, we examined 2 and 5 years of zoledronate treatment following adjuvant chemotherapy in the SUCCESS A trial.

Methods:
The SUCCESS A trial is a randomized, open-label, 2x2 factorial design Phase III study in high-risk early breast cancer patients that were randomized to adjuvant chemotherapy treatment with 3 cycles of FEC followed by either 3 cycles of docetaxel or 3 cycles of gemcitabine-docetaxel. After chemotherapy, patients were subject to a second randomization of 5 years of zoledronate treatment (4 mg i.v. every 3 months for 2 years, followed by 4 mg i.v. every 6 months for 3 years) vs. 2 years of zoledronate treatment (4 mg i.v. every 3 months for 2 years). Outcome was analyzed using adapted disease-free survival (DFS) and adapted overall survival (OS), with survival times measured as of 2 years after the start of zoledronate treatment. Maximal observation time was set to 4 years. Median observation time was 2.95 years for DFS and 3 years for OS.

Results:
Overall, 3421 of the 3754 patients randomized in the SUCCESS A study received at least one zoledronate dose. 434 patients had a DFS event or were lost to follow up in the first two years after the start of zoledronate treatment; thus, 2987 patients were available for analysis (1540 and 1447 patients in the 5-year and 2-year zoledronate treatment arm, respectively). Both adapted DFS and adapted OS did not differ between the two treatment arms (5 vs. 2 years) as shown by multivariate cox regressions adjusted for patient and tumor characteristics as well as chemotherapy (DFS: hazard ratio [HR] 0.97, 95% confidence interval [CI] 0.75 – 1.25, p = 0.81; OS: HR 0.98, 95% CI 0.67 – 1.42, p = 0.90). In addition, subgroup analyses according to menopausal status revealed no difference in DFS or OS between the two treatment arms in premenopausal women (DFS: HR 1.21, 95% CI 0.81 – 1.81, p = 0.35; OS: HR 0.93, 95% CI 0.57 – 1.53, p = 0.78) or postmenopausal women (DFS: HR 0.85, 95% CI 0.62 – 1.16, p = 0.30; OS: HR 0.96, 95% CI 0.67 – 1.39, p = 0.84).

Conclusions:
Our study showed no difference in DFS or OS between 5-years and 2-years of adjuvant zoledronate treatment in early breast cancer patients, irrespectively of menopausal status. Thus, our results indicate that extended treatment with zoledronate does not improve DFS or OS in high-risk early breast cancer patients.
Title: Sacituzumab govitecan (IMMU-132), an anti-Trop-2-SN-38 antibody-drug conjugate, as ≥3rd-line therapeutic option for patients with relapsed/refractory metastatic triple-negative breast cancer (mTNBC): efficacy results

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Body: Background: mTNBC has an aggressive course with limited therapeutic options. Sacituzumab govitecan (IMMU-132) is a novel antibody drug conjugate consisting of SN-38, the active metabolite of the topoisomerase I inhibitor, irinotecan, conjugated to a humanized mAb targeting Trop-2, which is highly expressed in most epithelial cancers, including TNBC. A phase I/II basket trial (NCT01631552) was conducted in patients (pts) with multiple, advanced epithelial cancers. We previously reported preliminary results in mTNBC (N=69; objective response rate [ORR] = 30%, Bardia et al., JCO 2017;35:2141-2148). In 2016, sacituzumab govitecan was granted Breakthrough Designation based on this encouraging data, and we resumed enrollment in a more defined patient population (≥3rd-line setting in mTNBC).

Methods: Pts received sacituzumab govitecan on days 1 & 8 of a 21-day cycle until progression or unacceptable toxicity. Eligibility included > 2 prior lines of therapy for metastatic disease, measurable disease by CT or MRI and prior taxane. Efficacy was assessed locally by RECIST 1.1 and confirmed by independent centralized blinded review. ORR, DOR, progression-free survival (PFS) and overall survival (OS) were determined. Adverse events (CTCAE v4.0), immunogenicity, and Trop-2 expression in archived tumor samples, when available, were evaluated.

Results: 110 mTNBC pts (109 female, 1 male; median age 55 yrs, range 31-81), including 53 from the previously reported cohort of 69 pts who had received ≥2 prior regimens for metastatic disease, were accrued between 7/2013 and 2/2017. As of data cutoff on 6/30/2017, 71 are deceased, 23 in long-term follow-up, and 16 still on treatment. All pts were treated at the 10 mg/kg IMMU-132 dose level, receiving 14.5 median doses (range 1-88). Treatment was well tolerated, with no treatment-related deaths, 2 treatment discontinuations for toxicity, and no anti-drug antibodies detected. Grade ≥3 toxicity (≥10%) included neutropenia, 39%; leukopenia, 14%; anemia, 10%; the incidence of febrile neutropenia was low (7%). By local radiologist assessment, the ORR is 34% (37/110), including 3 CRs and 34 PRs, the clinical benefit rate (CBR: CR+PR+SD>6 mo.) is 46%, the KM median DOR and PFS are 7.6 mo. (95% CI: 4.8 to 11.3) and 5.5 mo. (95% CI: 4.8 to 6.6), respectively, including 10% (11 pts) with long-term PFS (12 to 30+ mo.), and the KM median OS is 12.7 mo. (95% CI: 10.8 to 13.6). Results of the independent central blinded review along with sensitivity analyses of prior treatment regimens, including checkpoint inhibitor use, and exploratory biomarker analysis of Trop-2 expression will be presented at the meeting.

Conclusions: Sacituzumab govitecan demonstrated significant clinical activity as a single agent in the ≥3rd-line setting for patients with relapsed/refractory mTNBC. Given the high unmet medical need, data from this trial is being submitted for consideration of accelerated approval, and a global confirmatory randomized Phase III trial (NCT02574455) is underway. Additional studies including rational combinations are currently being evaluated for mTNBC and other breast cancer subsets.
Discovery and characterization of an estrogen bound LncRNA in late-Stage breast cancer

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Body: Breast cancer is the second most common newly diagnosed cancer and the second leading cause of cancer death among women in the United States. Despite the proven benefits of adjuvant endocrine therapy in women with hormone receptor positive breast cancer, relapses still occur over 5 years after initial treatment with endocrine therapy, referred to as late-stage relapse. Currently, the mechanisms of driving late-stage relapse are poorly characterized. To date, breast cancer research has primarily focused on protein-coding genes thereby missing the emerging class of long intergenic non-coding RNAs (lncRNAs) that may serve as critical regulators of relapse. Furthermore, the current understanding of lncRNA function is still in its infancy representing a critical gap for translating lncRNA discoveries into real-world applications to benefit patient care. Several well-described examples indicate that lncRNAs may be master epigenetic regulators in cancer biology through their interactions with proteins regulating target gene expression. Therefore, we hypothesize that lncRNAs may interact with estrogen receptor alpha 1 (ESR1) to regulate genes promoting late-stage relapse. To address this, we performed a transcriptome analysis of tumors from a unique cohort of 24 patients that had late-stage relapse to discover a novel set of lncRNAs, most of which have not yet been characterized. Next, we used RNA Immunoprecipitation coupled with transcriptome sequencing (RIP-Seq) to identify transcripts bound to ESR1 in T47D cells. We discovered 217 lncRNAs bound to ESR1 of which 50 were up-regulated in late-stage breast cancer. We chose to focus the most up-regulated lncRNA in late-stage relapse. Since it is an unannotated lncRNA we will refer to it as '\textit{LAte\text{-}Stage relapse ESR1\text{-}Bound LncRNA 1}', or LASER-1. To further understand the interplay between LASER-1 and ESR1, cells endogenously expressing LASER-1 were subjected to partial digestion to preserve lncRNA and protein interactions. Protected RNA fragments were subsequently immunoprecipitated with ESR1 and quantified by qPCR to reveal specific ESR1 interaction sites within LASER-1. Next, we observed increased expression of LASER-1 in ER\textsuperscript{+} breast cancer cell lines. Notably, LASER-1 expression was elevated in MCF7 long-term estrogen deprived (MCF7 LTED) cells -- that have amplified ESR1 -- relative to parental MCF7 cells. To demonstrate that LASER-1 promotes oncogenic phenotypes we transiently silenced LASER-1 in two cell lines with high endogenous expression of LASER-1 (including MCF LTED) and observed a decrease in cellular proliferation and invasion. Subsequent gene expression analysis after silencing LASER-1 altered mRNA and protein levels of critical cell cycle genes (i.e., p27). Overall, this is the first study to discover ESR1 bound lncRNAs that may be contributing to late-stage relapse in breast cancer. In the short-term, our ongoing research may lead to significant breakthroughs establishing the importance of LASER-1 as a master regulator in late-stage relapse. In the longer-term, we envision this research may lead to the development of novel therapeutics targeting LASER-1 with the potential for rapid clinical translation.
Body: Inactivating germline mutations in the NF1 gene (encoding neurofibromin) cause neurofibromatosis type 1. In addition to peripheral nervous system tumors, NF1 patients are at higher risk for other cancers, including breast cancer. Tumor exome-sequencing studies demonstrate that approximately 20% of all human cancers have somatic NF1 mutations. NF1 has been best known for its ability to inactivate Ras as a GAP (GTPase Activating Protein). However, this function is served by a small GAP domain in a very large protein. Recurrent missense mutations inactivating the GAP activity are infrequent. In contrast, it is common to detect frameshift (FS) and nonsense (NS) NF1 mutations, which can create an NF1-null state deleting not only GAP, but also, potentially, undefined NF1 functions whose loss could also drive tumorigenesis. As we reported at SABCS previously, in 600+ patients treated by tamoxifen adjuvant monotherapy, we found that FS/NS NF1 mutations independently correlate with relapse risk (HR=2.6, p=0.03). To explore this finding, we silenced NF1 in preclinical models of ER+ breast cancer, which markedly enhanced ER transcriptional activities, causing estradiol (E2) hypersensitivity and converted tamoxifen into an agonist (in vitro and in vivo). Most important, these activities depend on ER, but not on NF1’s GAP activity. These findings readily explain the poor patient outcomes associated with NS/FS NF1 mutations, and reveal a previously unrecognized function for NF1 in ER regulation.

In the presence of an agonist, liganded ER repels co-repressors and recruits co-activators, while the reverse is true with an antagonist such as tamoxifen. Many co-regulators contain leucine/isoleucine rich motifs, which bind directly to the ligand-binding domain (LBD) in ER. NF1 has several of these motifs that are much more highly conserved in species with a functional ER pathway, and some of these are mutated in cancers (e.g., in our patient cohort). Furthermore, we found that NF1 can bind directly to ER, and that this binding is mediated between the ER LBD and the NF1 leucine-rich regions. Like a classic co-repressor, wildtype NF1 (but not mutants lacking GAP activity or the Leu-rich motif) binds to ER, and is recruited by ER to the ERE in the presence of tamoxifen, but not E2. Further preclinical treatment studies indicate that while NF1-deficient ER+ breast cancer should not be treated by tamoxifen or AIs, fulvestrant remains effective. Furthermore, when fulvestrant is combined with dabrafenib and trametinib to inhibit Ras effectors Raf and MEK, apoptosis is induced in vitro, and tumor regression is observed in vivo. In conclusion, we have demonstrated that NF1 is a dual negative regulator at the intersection of two potent oncogenic signaling pathways, Ras and ER, and that NF1-deficient ER+ breast cancer patients may be more effectively treated by co-targeting the Ras and ER signaling. These patients, up to 10% of those with advanced ER+ breast cancer, can be readily identified for treatment by ctDNA analysis. A clinical trial is under development.
Title: Highly recurrent transcriptional remodeling events in advanced endocrine resistant ER-positive breast cancers

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Background: Although individual cancers are driven by heterogeneous processes, cancer mortality has a near universal cause—therapy resistance, recurrence and metastasis to vital organs. Characterizing more advanced tumors has borne valuable insight into cancer progression, yet studies of longitudinally collected breast cancer specimens are scarce given lengthy periods of cancer dormancy. In this study, we aimed to create the most comprehensive characterization of gene expression alterations to date between patient-matched pairs of primary and advanced ER-positive breast cancers.

Materials/Methods: Hybrid-capture RNA-sequencing was performed on 50 patient-matched pairs of primary and advanced ER-positive tumors from various recurrence sites (9 brain, 11 bone, 3 GI, 10 ovary, 17 local). Time to recurrence was up to 14.1 years with a median of 3.4 years. A shared variant analysis confirmed all paired samples were patient-matched. 1,380 cancer-related genes were analyzed for outlier expression fold-changes in matched recurrences versus primary tumors. Pair-specific, outlier fold-change thresholds were defined as Q1/Q3 +/- [1.5 X IQR]; using each pairs' fold-change values across all genes as the distribution. These discrete, longitudinal transcriptional remodeling events (LTREs) were assessed for recurrence across all sites and analyzed for enrichments within specific cohorts (Fisher's exact tests), such as locoregional vs. distant recurrences. To determine if LTREs represent acquired vulnerabilities, ex vivo and in vivo experiments targeting a recurrent, druggable LTRE gain of RET was performed.

Results: The majority of advanced cancers were transcriptionally similar to patient-matched primaries with 23 of 33 distant metastases retaining PAM50 assignments of the matched primary—shifts to HER2 (n=4, 12%) or Luminal B (n=5, 15%) subtypes accounted for most metastatic discordances. Despite this intrinsic conservation, remarkably recurrent gene-level LTRE gains and losses were observed in advanced disease. Recurrent LTRE gains included NCAM1 [42%], FGFR4 [40%], IBSP [36%], ROBO2 [36%] and SPP1 [30%]. Notable LTRE losses included RELN [42%] and ESR1 [26%]. NCAM1 LTREs showed the most significant enrichments (p < 0.001) in distant disease (20 of 33, 61%) versus locoregional disease (1 of 17, 6%). A prominent LTRE enriched in brain metastasis (BrM) was RET (p-value = 0.003), expression of which showed outlier gains in 56% of ER-positive BrMs. Marked anti-tumor activity was demonstrated with the RET inhibitor cabozantinib in ex vivo explant cultures of patient resected BrMs (n=3) and a BrM patient-derived xenograft.

Conclusions: Taken together, these results demonstrate profound, recurrent and metastatic site-specific LTREs in advanced breast cancers, which may be essential to our understanding of endocrine-therapy resistance and metastasis. Although current emphasis for longitudinal clinical profiling of tumors is on DNA-level alterations, these results suggest LTREs as a compelling, shared mechanism of cancer progression. Given remarkably high recurrence rates of specific LTREs across multiple cohorts, further preclinical and clinical investigations of LTREs are demanded, especially considering some (i.e. FGFR4 and RET) are readily druggable.
**2017 San Antonio Breast Cancer Symposium**

**Title:** First-line ribociclib vs placebo with goserelin and tamoxifen or a non-steroidal aromatase inhibitor in premenopausal women with hormone receptor-positive, HER2-negative advanced breast cancer: Results from the randomized phase III MONALEESA-7 trial

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**Body: Background:** Endocrine therapy (ET) with ovarian function suppression is an established first-line treatment for pre- and peri-menopausal women with hormone receptor-positive (HR+), human epidermal growth factor receptor 2-negative (HER2–) advanced breast cancer (ABC). Addition of ribociclib (orally bioavailable, selective cyclin-dependent kinase [CDK] 4/6 inhibitor) to first-line ET prolonged progression-free survival (PFS) in a Phase III trial of postmenopausal women with HR+, HER2– ABC (MONALEESA-2). Here we report results from MONALEESA-7 (NCT02278120), the first double-blind, randomized, Phase III trial evaluating ribociclib + tamoxifen/non-steroidal aromatase inhibitor (NSAI) and goserelin specifically in pre- and peri-menopausal patients.

**Methods:** Pre- or peri-menopausal women with HR+, HER2– ABC who had received ≤1 line of chemotherapy and no prior ET for ABC were randomized (1:1) to ribociclib (600 mg/day, 3-weeks-on/1-week-off) or placebo in combination with either tamoxifen (20 mg/day) or an NSAI (letrozole [2.5 mg/day] or anastrozole [1 mg/day]) + goserelin (3.6 mg every 28 days). The primary endpoint was locally assessed PFS. Secondary endpoints included overall response rate (ORR), clinical benefit rate (CBR), and safety.

**Results:** 672 patients were enrolled. Baseline patient characteristics were balanced between treatment arms. The primary analysis was conducted after 318 events had occurred; median time from randomization to data cut-off date was 19.2 months. The study met its primary objective: PFS was significantly improved in the ribociclib arm (median PFS = 23.8 months; 95% CI: 19.2–not reached) vs the placebo arm (median PFS = 13.0 months; 95% CI: 11.0–16.4), with a hazard ratio of 0.553 (95% CI: 0.441–0.694; p=9.83×10⁻⁶). Subgroup analyses demonstrated consistent PFS benefits for ribociclib vs placebo. In patients with measurable disease at baseline, ORR was 51% vs 36% (ribociclib vs placebo arm; p=3.17×10⁻⁴) and CBR was 80% vs 67% (p=3.40×10⁻⁴). The most frequent all-grade adverse events (Aes) ≥25% of patients; ribociclib vs placebo arm) were neutropenia (76% vs 8%), hot flush (34% vs 34%), nausea (32% vs 20%), leukopenia (31% vs 6%), and arthralgia (30% vs 27%). Of these, neutropenia (61% vs 4%) and leukopenia (14% vs 1%) were the only Grade 3/4 events reported in ≥5% of patients (ribociclib vs placebo arm). Febrile neutropenia (ribociclib vs placebo arm) occurred in 2% vs <1% of patients. Grade 3/4 QT prolongation (ribociclib vs placebo arm) was reported in 1% vs <1% of patients. Aes leading to permanent discontinuation of ribociclib + tamoxifen/NSAI + goserelin vs placebo + tamoxifen/NSAI + goserelin occurred in 4% vs 3% of patients.

**Conclusions:** MONALEESA-7, the first dedicated trial investigating a CDK4/6 inhibitor in pre- and peri-menopausal women with HR+, HER2– ABC, demonstrated that addition of ribociclib to first-line ET (tamoxifen/NSAI + goserelin) significantly prolonged PFS and had a manageable safety profile. The trial validates the clinical utility of ribociclib with multiple endocrine therapies, including tamoxifen, in premenopausal women with HR+, HER2– ABC.
Title: Phase Ib/II study evaluating safety and efficacy of pembrolizumab and trastuzumab in patients with trastuzumab-resistant HER2-positive metastatic breast cancer: Results from the PANACEA (IBCSG 45-13/BIG 4-13/KEYNOTE-014) study

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International Breast Cancer Study Group and Breast International Group.

Body: Background Preclinical and clinical data suggest that HER2-positive (HER2\(^+\)) breast cancer (BC) will be amendable to immunotherapeutic approaches. We evaluated pembrolizumab with trastuzumab in patients (pts) with trastuzumab-resistant HER2\(^+\), PD-L1 positive (PD-L1pos), unresectable loco-regional or metastatic BC and a parallel cohort of pts with HER2\(^+\), PD-L1 negative (PD-L1neg) BC during the phase II study.

Methods: Pts with advanced BC and progression on prior trastuzumab-based therapies, ECOG 0-1, and a metastatic tumor biopsy in the last year were eligible. HER2 positivity and quantity of tumor-infiltrating lymphocytes (TILs) on H&E slide were centrally evaluated. PD-L1 score was assessed by Merck central lab. Tumor imaging was performed at weeks 12, 18, 24 and every 12 weeks, thereafter. Primary endpoints were safety of the combination (phase Ib) and objective response rate (ORR) per RECIST 1.1 (phase II). Secondary endpoints were PFS, duration of response, and OS. Phase Ib was a 3+3 dose-escalation of 2 pembrolizumab doses (2mg/kg, 10mg/kg) Q3W. In phase II, pts received pembrolizumab 200mg Q3W for 24 months or until disease progression. Clinically stable pts with progression were allowed to continue pembrolizumab until confirmation on subsequent assessment. Pts with isolated CNS progression were also allowed to continue pembrolizumab after local treatment. Planned total enrollment was 61 pts. For the phase II PD-L1pos cohort, a Simon two-stage design (N=40; proceed if \(\geq\ 2/17\) respond) was used which had 85% power to compare ORR of 7% vs. 22% (1-sided \(\alpha=0.05\)). For the PD-L1neg cohort, a single-stage design with 15 pts had >95% power to compare ORR of 1% vs. 20% (1-sided \(\alpha=0.14\)). Clinicaltrials.gov: NCT02129556.

Results: 6 pts enrolled in phase Ib between April and July 2015; no DLTs were observed. The PD-L1pos cohort enrolled 40 pts between August 2015 and September 2016. The PD-L1neg cohort enrolled May 2016 to April 2017, stopping after 12 pts due to low rate of PD-L1 negativity, maintaining >90% power to detect the target difference in ORR. PD-L1 testing labs changed in April 2016. Prior to this time, QualTek PD-L1 positive was defined as \(\geq 1\)% on tumor or TILs. Using the Dako 22C3 antibody, positive was defined as tumor PD-L1 combined positive score (CPS)\(\geq 1\)%.

146 pts were screened to enroll 58 pts. Of screened pts, median stromal TILs was 1% (mean: 4.8%, SD: 9.1%, range: 0 to 60%; n=127); 52% of pts were PD-L1pos, with higher positivity rates while using the Dako assay compared with Qualtek (65% vs. 43%, p=0.009). Median TILs of pts in the PD-L1pos cohort was 2% (mean: 8.1%, SD: 11.2%, range: 0 to 40%) and 0% (mean: 1.2%, SD: 2.2%, range: 0 to 5%) in the PD-L1neg cohort.

Of enrolled pts, median age was 51yrs (range: 28-72), 69% had visceral metastases. 29% of pts received prior pertuzumab, 72% had prior T-DM1, 40% prior lapatinib. 38% of pts were ER-positive, 62% were ER-negative. Median TILs in enrolled ER pos and ER neg pts were 1.5% and 2.0%, respectively. PD-L1 positivity rates were also not significantly different by ER status (p=0.5). Final safety data and efficacy results will be presented at the meeting.
Title: MANTA - A randomized phase II study of fulvestrant in combination with the dual mTOR inhibitor AZD2014 or everolimus or fulvestrant alone in estrogen receptor-positive advanced or metastatic breast cancer

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Body: Background: Resistance to endocrine therapy remains a major clinical challenge with aberrant PI3K/ mTOR pathway activation being one of the main drivers. Randomised clinical trials have demonstrated a substantial benefit of adding everolimus to endocrine therapy. Vistusertib (AZD2014), a dual inhibitor of mTORC1 and mTORC2, has shown a broader range of activity in preclinical ER+ breast cancer models, showing superior activity to everolimus (EVE) both in hormone-sensitive and resistant models. The MANTA trial was designed to evaluate the safety and efficacy of vistusertib (VIS) in combination with fulvestrant (FULV) relative to FULV alone or FULV + EVE. In addition to a continuous (cont) daily schedule of VIS, the study also explored an intermittent (int) schedule to assess the potential of short-term, maximum target inhibition.

Methods: MANTA is an investigator-led, randomised, open-label phase II trial. Postmenopausal women with estrogen-receptor (ER)-positive breast cancer were eligible if they had disease recurrence while on or within 12 months of end of adjuvant treatment with an aromatase inhibitor (AI), or progression while on or within one month of end of AI treatment for locally advanced or metastatic breast cancer. Patients were randomly assigned (2:3:3:2) to receive either FULV (500 mg intramuscular injection on day 1, followed by 500 mg doses on days 15 and 29, and then every 28 days); FULV + daily VIS (50mg BD), FULV + intermittent VIS (2 days on, 5 days off; 125mg BD); or FULV + EVE (10mg OD). Treatment was given until disease progression (RECIST 1.1) or intolerable toxicity. Patients were stratified by disease measurability and response to prior endocrine therapy. The primary endpoint was investigator-assessed progression-free survival (PFS). Secondary objectives included objective response, clinical benefit rate, duration of response and clinical benefit, overall survival and safety.

Results: Between 04/2014 and 10/2016, a total of 333 patients were randomised at 88 sites in 9 countries. 66 patients were assigned to receive FULV; 101 to FULV+VIS (cont), 95 to FULV+VIS (int); and 64 to FULV+EVE. Median PFS was 4.6 months (95% CI 3.4–6.9) in patients assigned to FULV; 7.5 months (95% CI 5.6–9.4) in those assigned to FULV+VIS (cont); 7.6 months (95% CI 5.5–9.6) in those assigned to FULV+VIS (int); and 12.2 months (95% CI 7.5–14.3) in those assigned to FULV+EVE. No significant difference was recorded between the patients assigned to FULV+VIS (cont) and FULV (hazard ratio 0.87, 95% CI 0.62–1.23; log-rank p=0.42); FULV+VIS (int) and FULV (HR 0.78, 95% CI 0.55–1.12; log-rank p=0.16); and FULV+VIS (cont) and FULV+VIS (int) (HR 1.11, 95% CI 0.81–1.52; log-rank p=0.52). PFS was significantly longer in patients assigned to FULV+EVE compared to FULV+VIS (cont) (HR 0.64, 95% CI 0.45–0.91; log-rank p=0.01) and FULV+EVE compared to FULV (HR 0.64, 95% CI 0.43–0.94; log-rank p=0.02).

Conclusion: The trial failed to demonstrate a benefit of adding the TORC1/2 inhibitor vistusertib (AZD2014) to FULV. The combination FULV+EVE demonstrated significantly longer PFS compared to FULV+VIS or FULV.
Title: A prospective randomized multi-center phase-III trial of additional 2 versus additional 5 years of anastrozole after initial 5 years of adjuvant endocrine therapy – results from 3,484 postmenopausal women in the ABCSG-16 trial

Body: Background: While extended adjuvant therapy with aromatase inhibitors (AI) after initial tamoxifen has been demonstrated to improve disease-free-survival (DFS) of postmenopausal patients with hormone-receptor positive breast cancer, the optimal duration of extended AI is unknown. Moreover, it remains unclear whether patients after AI in the first 5 years benefit similarly from extended adjuvant AI therapy as patients after Tamoxifen.

Methods: From February 2004 to June 2010, 3484 women with postmenopausal stage I-III hormone-receptor positive early breast cancer were randomized in 71 centers in Austria to receive either 2 years or 5 years of additional Anastrozole (1 mg daily) as extended adjuvant therapy, after initial 5 years of adjuvant endocrine treatment. Eligible patients had to be recurrence-free at 60 months of initial adjuvant therapy with Tamoxifen (Tam) or AI or Tam→AI, and younger than 80 years of age. Stratification factors were tumor stage, nodal status, initial endocrine therapy, adjuvant chemotherapy, and quantitative hormone receptors. Patients were followed-up at least annually. Primary end point of ABCSG-16 was DFS, secondary end points included overall survival (OS), fractures, contralateral breast cancer, and toxicity.

Results: As of June 30, 2016, the median follow-up of the 3468 patients included in the analysis of ABCSG-16 was 105.9 months (IQR 102.2-110.3 months) after randomization (i.e. approx. 14 years after diagnosis). Median patient age was 64 years, 2507 (72%) patients had tumors smaller than 2 cm, 2301 (66%) patients were node-negative, 674 (19%) patients had high-grade tumors, 2683 (77%) patients had tumors both ER and PR positive. 2764 (80%) patients were treated with breast conserving surgery. Before randomization into ABCSG-16, 1000 (29%) patients had undergone (neo)adjuvant chemotherapy, 1774 (51%) patients had received 5 years of Tamoxifen, whereas 1688 (49%) patients had received other (AI containing) regimens in the first five years.

As of June 30, 2016, 757 DFS events have been recorded, 377 (22%) in the 2-year group, and 380 (22%) in the 5-year group. There was no significant difference in DFS (HR 0.997, 95%CI 0.86-1.15, log rank p=0.982), in OS, time to secondary carcinoma and time to contralateral breast cancer. With respect to drug adherence, 81.2% of patients in the 2-year arm were taking the study drug still at 2 years, and 80.1% at 2 years in the 5-year arm. At 5 years, 65.6% of patients in the 5-year arm were still on the assigned medication. Bone fractures were more frequent in the 5-year arm (i.e. years 3 to 5 after randomization: 6% vs 4 %, HR=1.405, 95%CI 1.03-1.91, p=0.029).

Conclusion: After 5 years of adjuvant endocrine therapy (Tamoxifen or AI or Sequence), 2 additional years of Anastrozole are sufficient for extended adjuvant therapy – a further extension to 5 additional years did not yield additional outcome benefit but added toxicity.

Support: AstraZeneca
**Title:** Invasive disease-free survival and gene expression signatures in CALGB (Alliance) 40601, a randomized phase III neoadjuvant trial of dual HER2-targeting with lapatinib added to chemotherapy plus trastuzumab

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**Body:**

**Purpose**

Dual HER2 targeting increases pathologic complete response (pCR) rate to neoadjuvant therapy and improves outcomes in both early and metastatic HER2-positive disease. CALGB 40601 is a randomized phase III trial examining the impact of dual HER2 blockade consisting of trastuzumab (H) and lapatinib (L) added to paclitaxel (T) on pCR, considering tumor and microenvironment molecular features. We previously found that pCR was numerically but not significantly increased with dual therapy, and that tumor molecular subtype and evidence of immune activation significantly and independently affected pCR (Carey et al, JCO 2016). In this secondary analysis, we sought to evaluate the effects of treatment arm and gene expression-defined subgroups on invasive disease free survival (IDFS).

**Patients and Methods**

Patients (Pts) with stage II to III HER2-positive breast cancer underwent tumor biopsy followed by random assignment with equal probability paclitaxel plus trastuzumab alone (TH) or with the addition of lapatinib (THL) for 16 weeks before surgery. A paclitaxel plus lapatinib (TL) arm was closed early based on reports of futility from other trials. A secondary endpoint was IDFS, defined as the time from surgery until local or distant recurrence, new primary, or death from any cause, whichever was first. Gene expression signatures were identified by RNA sequencing.

**Results**

Between 12/2008 and 2/2012, 305 pts were enrolled. 261 pts had IDFS and gene expression information available (THL, n = 103; TH, n =101; TL, n = 57); there were no significant differences in clinical characteristics between this subset and the entire population. The median IDFS follow-up was 4.6 years with 40 IDFS events having occurred (THL, n=7; TH, n=19; TL, n=14). IDFS was significantly longer in the THL arm compared to standard TH (HR=0.34; 95% CI: 0.14-0.82; p=0.02). IDFS was also significantly longer among pCR than non-pCR pts (HR=0.40; 95% CI: 0.19-0.81; p=0.01), and did not differ by hormone receptor (HR) status, clinical stage, tumor size, race, menopausal status or age. Among gene expression signatures, only immune activation measured by an IgG signature was associated with longer IDFS (HR=0.71; 95% CI: 0.51-0.98; p=0.04); this signature was previously also associated with pCR. Multivariate analysis showed dual therapy (HR=0.35; p=0.02), pCR (HR=0.36; p=0.01), IgG (HR=0.69; p=0.05), and molecular subtype (LumA vs HER2E, HR=0.24, p=0.005) were associated with longer IDFS. A subgroup analysis by hormone receptor status revealed that among pts with HR+ disease, pts with luminal A experienced longer IDFS (HR=0.23; p=0.02) compared to those with luminal B or HER2-enriched molecular subtypes.

**Conclusion**

Dual HER2-targeting with lapatinib added to 16 weeks of TH produced significantly longer IDFS than TH alone, despite modest effects on pCR. Similar to pts with HER2-negative disease, pts with luminal A had better IDFS than those with other molecular subtypes. Immune activation as measured by RNA-based signature independently predicted both pCR and IDFS.

**Support:** U10CA180882, U10CA180821, U24CA196171, P50-CA58823, Susan G Komen, BCRF
Title: A phase III multicentre double blind randomised trial of celecoxib versus placebo in primary breast cancer patients (REACT – Randomised EuropeAn celecoxib trial)

R C Coombes1, Holly Tovey2, Lucy Kilburn3, Janine Mansi4, Carlo Palmieri4, John Bartlett4, Johnathon Hicks6, Andreas Makris7, Abigail Evans8, Sibylle Loibl8, Carsten Denkert9, Elisabeth Murray11, Robert Grieve12, Robert Coleman13, Marcus Schmidt19, Peter Klare14, Mahdi Rezai15, Beate Rautenberg16, Nicole Klutinus17, Uwe Rhein18, Kelly Mousa1, Susana Ricardo-Vitorino1, Gunter von Minckwitz2 and Judith Bliss2. 1Imperial College London, UK; 2Institute of Cancer Research - Clinical Trials and Statistics Unit, UK; 3Guys’ & St Thomas’ NHS Foundation Trust and Biomedical Research Centre, King’s College London, UK; 4University of Liverpool and Clatterbridge Cancer Centre, UK; 5Ontario Institute for Cancer Research, Toronto, Canada; 6NHS Lanarkshire, UK; 7Mount Vernon Cancer Centre, UK; 8Poole Hospital NHS Foundation Trust, UK; 9German Breast Group, Neu-Isenburg, Germany; 10Charité University Hospital and German Cancer Consortium (DKTK), Berlin, Germany; 11United Lincolnshire Hospitals NHS Foundation Trust, UK; 12University Hospitals Coventry and Warwickshire NHS Trust, UK; 13University of Sheffield. Sheffield, UK; 14Praxisklinik Krebsheilkunde, Berlin, Germany; 15Luisenkrankenhaus Düsseldorf, Germany; 16Universitätsklinikum Freiburg, Germany; 17Klinikum Pforzheim GmbH, Germany; 18SRH Zentralklinikum Suhl GmbH, Germany and 19Universitatsmedizin Mainz, Germany.

Body: Background
Inhibition of COX-2 has been shown to attenuate the metastatic process in pre-clinical models of human breast cancer (BC). The primary aim of this study was to assess the effect of 2 years adjuvant therapy with the COX-2 inhibitor celecoxib compared with placebo in HER2-ve primary BC patients.

Patients & Methods
Patients were randomised in a 2:1 ratio to receive celecoxib 400mg once daily or placebo for 2 years. Patients had to have completely resected BC with prior local and systemic adjuvant treatment according to local practice. Concurrent radiotherapy was permitted and hormone receptor +ve patients received endocrine therapy according to local practice. Patients with HER2+ or node negative, T1 and grade 1 disease were excluded. Median age of patients was 55 years (IQR: 49-63). 50% of patients had tumours >2cm; 42% were grade 3; 48% had node +ve disease. According to local assessment 73% were ER/PgR +ve. Primary endpoint was Disease Free Survival (DFS); defined as time from randomisation to date of first event, with events contributing to analysis defined as recurrence (distant/local), new primary BC (ipsilateral/contralateral) and death. Secondary endpoints included Overall Survival (OS), toxicity, cardiovascular mortality and incidence of second primaries. Subgroup analysis by hormone receptor status was pre-planned. Survival endpoints are analysed using Cox-proportional hazards and log-rank tests; restricted mean survival is used where proportional hazards do not hold.

Results
Between January 2007 and November 2012, 2639 patients were randomised (1763 celecoxib; 876 placebo) from 181 centres across the UK and Germany. At 13th April 2017, median follow up was 60 months (IQR: 48-72) with 428 DFS events reported. Unadjusted survival analysis results are presented below, with hazard ratio<1 favouring celecoxib:

<table>
<thead>
<tr>
<th></th>
<th>5 year survival estimate (95% CI)</th>
<th>Hazard ratio (95% CI)</th>
<th>p-value</th>
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<tbody>
<tr>
<td><strong>DFS (all patients)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Celecoxib</td>
<td>83% (81, 85)</td>
<td>1.02 (0.83 – 1.24)</td>
<td>0.88</td>
</tr>
<tr>
<td>Placebo</td>
<td>83% (80, 86)</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td><strong>DFS within ER+</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Celecoxib</td>
<td>87% (85, 89)</td>
<td>0.89 (0.69 – 1.16)</td>
<td>0.40</td>
</tr>
<tr>
<td>Placebo</td>
<td>86% (83, 89)</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>DFS within ER-</td>
<td></td>
<td>OS (all patients)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Celecoxib</td>
</tr>
<tr>
<td>Celecoxib</td>
<td>72% (68, 76)</td>
<td>1.17 (0.85 – 1.61)</td>
<td>90% (88, 91)</td>
</tr>
<tr>
<td>Placebo</td>
<td>75% (69, 80)</td>
<td>1</td>
<td>Placebo</td>
</tr>
</tbody>
</table>

The interaction between ER status and treatment was not significant; p=0.36.

In the celecoxib and placebo groups there were 17 and 8 deaths respectively in patients who had not relapsed. These were due to cardiac (n=3; 2) and other (n=14; 6) in the celecoxib and placebo groups respectively; none were GI related. In total 304 serious adverse events were observed in 265 patients (186/1763 celecoxib; 79/876 placebo). In the celecoxib and placebo groups respectively these were related to cardiac (n=12; 7), GI (n=9; 2) and other (n=193; 81). Work is ongoing to determine whether a subset of ER+ patients whose primary tumours show the characteristics of a COX-2 signature receive greater benefit from celecoxib.

**Conclusions**

There is no benefit of celecoxib in the ITT population. Further exploratory studies focussing on the ER+ subpopulation are ongoing. Celecoxib treatment is not associated with significant toxicity when compared to placebo in this population of BC patients.
Title: A randomized phase III study of adjuvant trastuzumab for a duration of 9 weeks versus 1 year, combined with adjuvant taxane-anthracycline chemotherapy, for early HER2-positive breast cancer (the SOLD study)


1Helsinki University Hospital and University of Helsinki, Helsinki, Finland; 2Beatson West of Scotland Cancer Centre, Glasgow, United Kingdom; 3University Hospitals Leuven, Leuven, Belgium; 4Turku University Central Hospital, Turku, Finland; 5Kuopio University Hospital, Kuopio, Finland; 6Helsinki University Hospital and University of Helsinki, Helsinki, Finland; 7Päijät-Häme Central Hospital, Lahti, Finland; 8Örebro University Hospital, Örebro, Sweden; 9Helsinki University Hospital and University of Helsinki, Helsinki, Finland; 10Västerås Central Hospital, Västerås, Sweden; 11Skåne University Hospital, Lund, Sweden; 12Eskilstuna Hospital, Eskilstuna, Sweden; 13Oulu University Hospital, Oulu, Finland; 14Beatson West of Scotland Cancer Centre, Glasgow, United Kingdom; 154Pharma, Turku, Finland; 16University Hospitals Leuven, Leuven, Belgium; 17Beatson West of Scotland Cancer Centre, Glasgow, United Kingdom; 18Auckland City Hospital, Auckland, New Zealand; 19Tampere University Hospital, Tampere, Finland and 20Uppsala University Hospital, Uppsala, Sweden.

Body: Background: The optimal duration of trastuzumab (T), when given together with chemotherapy and after chemotherapy as adjuvant treatment in patients with HER2+ breast cancer (BC), is unknown. Whilst the international standard is 12 months of T, the benefits and harms of T treatment continued beyond the chemotherapy are unclear.

Methods: Women with histologically confirmed node-negative or node-positive HER2+ BC were eligible for the trial (NCT00593697). The primary tumor diameter was required to be >5 mm in node-negative cancer. Patients with distant metastases, inflammatory cancer, clinically significant cardiac disease, left ventricular ejection fraction (LVEF) <50%, unknown estrogen receptor (ER) status, World Health Organization performance status >1, and those who had received neoadjuvant systemic cancer therapy were excluded. Patients were randomly assigned to 2 groups prior to starting systemic cancer therapy. The initial systemic treatment was identical in the groups consisting of 3 cycles of 3-weekly docetaxel plus T followed by 3 cycles of fluorouracil, epirubicin, and cyclophosphamide (FE75C). Thereafter, no further T or chemotherapy was administered in Arm A, whereas in Arm B single-agent T was administered 3-weekly for 14 cycles to complete 1 year of T treatment. The docetaxel dose was either 80 mg/m2 or 100 mg/m2 (prespecified for each center). Radiation therapy and endocrine therapy (for patients with ER+ cancer) were given according to the institutional practice; the minimum scheduled duration of endocrine therapy was 5 years. The LVEF was measured pretreatment, and on study weeks 18, 31, 43, and 61 and month 36. The primary endpoint was disease-free survival (DFS) compared between the groups using a Cox model and the non-inferiority approach.

Results: A total of 2,176 patients were entered into the study from 63 centers in 7 countries from Jan. 3, 2008 to Dec.16, 2014. The median follow-up time was 5.2 years at data collection closure (Dec. 31, 2016). The efficacy and safety data will be presented at the meeting.
Title: Survival analysis of the prospectively randomized phase III GeparSepto trial comparing neoadjuvant chemotherapy with weekly nab-paclitaxel with solvent-based paclitaxel followed by anthracycline/cyclophosphamide for patients with early breast cancer – GBG69

Body: Introduction
The GeparSepto study showed that the substitution of paclitaxel (P) with nab-paclitaxel (nP) followed by epirubicin/cyclophosphamide (EC) as neoadjuvant chemotherapy increased the rate of pathological complete response (pCR) from 29% to 38% (p<0.001). A pronounced improvement of pCR from 26% to 48% (OR: p<0.001) was achieved in patients with triple-negative BC (TNBC) (Untch et al. Lancet Oncology 2016). It has not yet been shown whether these effects on pCR will be translated into a survival benefit. We here report the survival analysis of the GeparSepto trial.

Patients and Methods
In the GeparSepto trial (NCT01583426) patients were randomized in a 1:1 ratio to receive either nP 125mg/m² or P 80 mg/m² q1w for 12 weeks followed by 4 cycles of conventionally dosed EC (E: 90mg/m²; C: 600 mg/m²) q3w (Furlanetto et al. Annals Oncol 2016). Patients with HER2+ tumors received trastuzumab 6(8)mg/kg q3w and pertuzumab 420(840)mg q3w concomitantly to all chemotherapy cycles (Loibl et al. Annals Oncol 2016). Patients with untreated, histologically confirmed uni- or bilateral, cT2-cT4d breast carcinoma, and no clinically relevant cardiovascular and other co-morbidities were included. Primary objective was pCR rate (ypT0 ypN0). Secondary objectives were invasive disease-free survival (IDFS), and overall survival (OS) overall and according to stratified subpopulations, amongst other time to event endpoints, quality of life focusing on peripheral sensory neuropathy (PNP), treatment of PNP, and cardiac toxicity, detection of circulating tumor (ct) DNA at the time of surgery and during follow up and correlation with pCR and early relapses. The IDFS analysis is planned after 248 events have occurred. The log-rank test will have 80% power to detect an improvement of the 5 year IDFS from 75% to 81.8% (HR=0.70) at a 2-sided significance level of \(\alpha=0.05\).

Results
In 69 German centers, 1229 patients were randomly assigned (07/2012 – 12/2013) to receive either nP (606) or P (600). nP was given for the majority of cycles at a dose of 150mg/m² to 179 patients and at a dose of 125mg/m² to 426 patients. Follow-up is still ongoing. The expected number of events will be awaited for October 2017.

Conclusion
Neoadjuvant GeparSepto study demonstrated a significantly higher pCR rate when patients received nP instead of P as part of an anthracycline/taxane based sequential chemotherapy. The expected long-term results will help to assess the overall benefit of nP in BC and the surrogate value of pCR for survival endpoints.
Title: Long-term follow-up of CALGB 40502/NCCTG N063H (Alliance): A randomized phase III trial of weekly paclitaxel (P) compared to weekly nanoparticle albumin bound nab-Paclitaxel (NP) or ixabepilone (Ix) +/- bevacizumab as first-line therapy for locally recurrent or metastatic breast cancer (MBC)


Body: Background: CALGB 40502/NCCTG N063H (Alliance) compared weekly NP or Ix to P; most patients received bevacizumab. Ix was inferior to P, and NP was not superior with a trend toward inferiority. Toxicity was increased in the experimental arms compared to P (Rugo et al, JCO 2015). We report long-term follow-up (FU) of this trial with an unplanned subset analysis in hormone receptor positive (HR+) and triple negative (TNBC) breast cancer.

Methods: Patients were randomized 1:1:1 to receive P (90 mg/m²), Ix (16 mg/m²) or NP (150 mg/m²) on a 3 week (wk) on, 1 wk off schedule, stratified by prior adjuvant taxane use and hormone receptor status. B was initially given to all patients, but became optional in 3/2011 and was added to stratification. The primary endpoint was progression-free survival (PFS); secondary endpoints included safety and overall survival (OS). With a target N=900 patients, the study was powered to detect a hazard ratio of 1.36 (median PFS 10 vs 13.6 months). Eligibility included no prior chemotherapy for MBC, >12 mo from adjuvant P and measurable disease.

Results: 799 patients were randomized between 11/08 and 11/11 (283 to P, 271 to NP, 245 to Ix); 98% received bevacizumab. 68% (546) had HR+ disease, 25% (201) had TNBC. Median FU is 5 years. Median PFS is unchanged at 10.8, 9.2 and 7.4 mo for P, NP and Ix with hazard ratios (95% CIs) of 1.13 (0.94-1.34) and 1.44 (1.2-1.72) for NP and Ix to P, respectively. Median OS was 27.1, 24.2 and 23.6 months for P, NP and Ix with hazard ratios of 1.28 (0.9-1.82) and 1.35 (1.07-1.71) for NP and Ix to P, respectively. The effects of NP vs P on PFS and OS were significantly modified by subtype (interaction p=0.0018 and 0.0073), whereas Ix vs P was unchanged (interaction p's > 0.9, Table). More patients discontinued treatment due to adverse events in the experimental arms (14 vs 27 vs 23% for P, NP and Ix).

<table>
<thead>
<tr>
<th></th>
<th>P (mo)</th>
<th>NP (mo)</th>
<th>NP to P; HR (95% CI)</th>
<th>Ix (mo)</th>
<th>Ix to P, HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNBC, PFS</td>
<td>6.4</td>
<td>7.4</td>
<td>0.79 (0.55-1.12)</td>
<td>5.6</td>
<td>1.39 (0.99-1.96)</td>
</tr>
<tr>
<td>HR+, PFS</td>
<td><strong>12.2</strong></td>
<td>9.6</td>
<td>1.29 (1.04-1.59)</td>
<td><strong>8</strong></td>
<td>1.5 (1.21-1.86)</td>
</tr>
<tr>
<td>TNBC, OS</td>
<td>15.3</td>
<td><strong>21</strong></td>
<td><strong>0.74 (0.51-1.07)</strong></td>
<td>15.1</td>
<td>1.28 (0.9-1.82)</td>
</tr>
<tr>
<td>HR+, OS</td>
<td>33.2</td>
<td>26.6</td>
<td>1.25 (0.99-1.58)</td>
<td>25.4</td>
<td>1.35 (1.07-1.71)</td>
</tr>
</tbody>
</table>

Interaction tests: 1. p=0.0018; 2. p=0.0073; 3. p=0.96; 4. p=0.92 mo: months; HR: hazard ratio

Conclusion: In patients with chemotherapy-naive MBC, Ix was inferior to P for PFS, and P was better tolerated than either NP or Ix. In this retrospective subset analysis, Ix and NP were inferior to P in HR+ disease, with a suggestion of improved PFS and OS with NP in patients with TNBC. Further investigation is required to explain and validate the subtype specificity seen in this exploratory analysis.

Support: U10CA180820, U10CA180821, U10CA180882, U10CA180888. ClinicalTrials.gov Identifier: NCT00785291
2017 San Antonio Breast Cancer Symposium

Publication Number: GS3-07

Title: Genome-wide copy number analysis of chemotherapy-resistant metastatic triple-negative breast cancer from cell-free DNA

Daniel G Stover¹, Heather A Parsons², Gavin Ha³, Sam Freeman³, Bill Barry², Hao Guo², Atish Choudhury², Greg Gydush³, Sarah Reed³, Justin Rhoades³, Denisse Rotem³, Melissa E Hughes², Deborah A Dillon², Ann H Partridge², Nikhil Wagle²,³, Ian E Krop², Gad Getz³, Todd A Golub², J Christopher Love³, Eric P Winer², Sara M Tolaney³, Nancy U Lin² and Viktor A Adalsteinsson³. ¹The Ohio State University Comprehensive Cancer Center, Columbus, OH; ²Dana-Farber Cancer Institute, Boston, MA; ³Broad Institute of Harvard and MIT, Cambridge, MA and ⁴Massachusetts Institute of Technology, Cambridge, MA.

Body: Introduction:
Triple-negative breast cancer (TNBC) is a poor prognosis breast cancer subset characterized by relatively few mutations but extensive copy number alterations (CNAs). Cell-free DNA (cfDNA) offers the potential to overcome infrequent tumor biopsies in metastatic TNBC (mTNBC) and interrogate the genomics of chemotherapy resistance.

Methods:
506 archival or fresh plasma samples were identified from 164 patients with mTNBC who had previously received chemotherapy. We performed low coverage whole genome sequencing to determine genome-wide copy number and estimate 'tumor fraction' of cfDNA (TFx) using our recently-developed approach, ichorCNA. In patient samples with TFx >10%, we identified regions that were significantly gained or lost using GISTIC2.0. We compared CNAs of 20 paired primary-metastatic samples and also mTNBCs from cfDNA versus primary TNBCs from TCGA and METABRIC.

Results:
We successfully obtained high quality, low coverage whole genome sequencing data for 478 (94.5%) plasma samples from 158 patients, with 1 to 14 samples per patient. TFx and copy number profiles were highly concordant with paired metastatic biopsy (n=10, range 0-7 days from biopsy to blood draw) with sensitivity of 0.86 and specificity of 0.90 and reproducible in independently-processed blood draws (TFx intraclass correlation coefficient 0.984). Median overall survival from time of first blood draw was 8 months, and TFx was highly correlated independent of primary stage, primary receptor status, age at primary diagnosis, BRCA status, and metastatic line of therapy: adjusted hazard ratio between 4th and 1st quartiles = 2.14 (95% CI 1.40-3.28; p=0.00049). 101/158 patients (63.9%) had at least one sample with TFx >10%, our threshold for high confidence CNA calls. Copy number profiles and percent genome altered were remarkably similar between mTNBCs and primary TNBCs in TCGA and METABRIC (n=433), suggesting that large-scale chromosomal events are infrequent in TNBC metastatic progression. We identified chromosomal gains that demonstrated significant enrichment in mTNBCs relative to paired primary TNBCs (n=20) and also TCGA/METABRIC, including driver genes (NOTCH2, AKT2, AKT3) and putative antibody-drug conjugate targets. Finally, we identify a novel association of gains of 18q11 and/or 19p13 with poor metastatic prognosis, independent of clinicopathologic factors and TFx.

Conclusions:
Here, we present the first large-scale genomic characterization of metastatic TNBC to our knowledge, derived exclusively from cfDNA. 'Tumor fraction' of cfDNA is an independent prognostic marker in mTNBC. Primary and metastatic TNBC have remarkably similar copy number profiles yet we identify alterations enriched and prognostic in mTNBC. Collectively, these data have potential implications in the understanding of metastasis, therapeutic resistance, and novel therapeutic targets.
**2017 San Antonio Breast Cancer Symposium**

**Publication Number:** GS3-08

**Title:** Pathological complete response predicts event-free and distant disease-free survival in the I-SPY2 TRIAL

Douglas Yee¹, Angela DeMichele², Claudine Isaacs³, Fraser Symmans⁴, Christina Yau⁵, Kathy S Albain⁶, Nola M Hylton⁷, Andres Forero-Torres⁸, Laura J van't Veer⁹, Jane Perlmutter⁹, Hope S Rugo⁵, Michele Melisko⁵, Yunn-Yi Chen¹, Ron Balassanian⁵, Gregor Kriangs⁵, Brian Datnow⁹, Farnaz Hasteh⁹, Anne Tipps⁹, Noel Weidner⁹, Hong (Amy) Zhang², Ronald Tickman¹⁰, Sean Thornton¹⁰, Jon Ritter¹, Khalid Amin¹, Molly Klein¹, Beiyun Chen¹¹, Gary Keeney¹¹, Tolgay Ocal¹², Mike Feldman², Nancy Klipfel¹³, Husain Sattar¹⁴, Jeffery Mueller¹⁴, Katja Gwin¹⁴, Gabrielle Baker¹⁴, Bhaskar Kallakury¹, Jay Zeck³, Xiuze Duan⁵, Cagatay Ersahin⁶, Roberto Gamez⁶, Megan Troxell¹⁵, Atiya Mansoor¹⁵, Lauren Grasso LeBeau¹⁶, Sharon Sams¹⁷, Josh Wisell¹⁷, Shi Wei⁷, Shuko Harada¹, Tuyethoa Vinh¹⁸, Michael D Stamatakos¹⁸, Ossama Tawfik¹⁹, Fang Fan¹⁹, Amy Adams²⁰, Mara Rendi²¹, Susan Minton²², Anthony Magliocco²², Sunati Sahoo²³, Yisheng Fang²³, Gillian Hirst³, Ruby Singhrao³, Smita M Asare²⁴, Anne M Wallace⁹, A J Chien⁹, Erin D Ellis¹⁰, Heather S Han²², Amy S Clark², Judy C Boughhey¹¹, Anthony D Elias¹⁷, Rita Nanda¹⁴, Larissa Korde³¹, Rashmi Murthy⁵, Julie Lang¹³, Donald Northfelt¹³, Qamar Khan¹⁹, Kirsten K Edmiston¹⁹, Rebecca Viscusi¹⁶, Barbara Haley³¹, Kathleen Kemmer¹⁵, Amelia Zelnak²⁰, Donald A Berry⁴ and Laura J Esserman⁶. ¹University of Minnesota, Minneapolis, MN; ²University of Pennsylvania, Philadelphia, PA; ³Georgetown University, Washington, DC; ⁴University of Texas, M.D. Anderson Cancer Center, Houston, TX; ⁵University of California, San Francisco, San Francisco, CA; ⁶Loyola University, Maywood, IL; ⁷University of Alabama at Birmingham, Birmingham, AL; ⁸Gemini Group, Ann Arbor, MI; ⁹University of California, San Diego, San Diego, CA; ¹⁰Swedish Cancer Institute, Seattle, WA; ¹¹Mayo Clinic, Rochester, Rochester, MN; ¹²Mayo Clinic, Scottsdale, Scottsdale, AZ; ¹³University of Southern California, Los Angeles, CA; ¹⁴The University of Chicago Medical Center, Chicago, IL; ¹⁵Oregon Health and Science University; ¹⁶University of Arizona; ¹⁷University of Colorado, Denver; ¹⁸Inova Health System; ¹⁹University of Kansas; ²⁰Emory University; ²¹University of Washington; ²²Moffitt Cancer Center; ²³University of Texas, Southwestern and ²⁴Quantum Leap Health Care Collaborative.

**Body:** Background: Pathological complete response (pCR) is accepted by FDA as a surrogate endpoint for accelerated approval of targeted agents in combination with chemotherapy based on better long-term outcomes compared to residual disease (Cortazar 2014).

**Methods:** The multi-center, adaptively-randomized I-SPY2 platform trial uses pCR as the primary endpoint to identify investigational agents that will improve outcomes in women with stage 2/3 breast cancer with high risk of early recurrence, across all signatures, based on hormone receptor (HR), HER2, and 70-gene (MammaPrint) status. For patients with HR+ HER2- tumors, only 70-gene (MammaPrint) high-risk patients are enrolled. To date, 1200+ patients have been randomized to one of 14 arms: control (paclitaxel followed by AC); veliparib/carboplatin; neratinib; MK2206; trebananib; trastuzumab/pertuzumab; ado-trastuzumab emtansine/pertuzumab; pembrolizumabx4; ganitumab/metformin; ganetespib; PLX-3397. 7 agents graduated in the first 522 pts (median follow-up:2.5 years). 180 pts achieved pCR (36%) while 338 did not (RCB=1-3). There were 82 EFS and 65 DRFS events. Over the entire group (including all arms), pCR was highly associated with 3-year EFS (p<0.001 for both). Pts achieving pCR had a 3% recurrence risk (RR) at 3 years; those with non-pCR had 24% RR over this time period. For distant recurrence, the 3-year RR with pCR was 2%, compared to 20% in pts with non-pCR. As expected, pCR rates varied by breast cancer subtype (HR+/HER2: 18% (35/196), HR+/HER2+: 40% (33/82), HR-/HER2+:68% (34/50), HR-/HER2-:41% (76/188)). The relationship between pCR and EFS was significant and clinically impactful within each subtype.

<table>
<thead>
<tr>
<th></th>
<th>3-year survival (pCR group)</th>
<th>Hazard Ratio</th>
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<tbody>
<tr>
<td></td>
<td>Overall</td>
<td>Overall</td>
</tr>
<tr>
<td>EFS</td>
<td>97%</td>
<td>0.08 (0.03-0.23)</td>
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<tr>
<td>DDFS</td>
<td>98%</td>
<td>0.08 (0.03-0.26)</td>
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</tbody>
</table>

**Conclusions:** The first long-term efficacy results from the I-SPY2 TRIAL demonstrate that achieving pCR is a very strong surrogate endpoint for improved EFS and DDFS in a high-risk population, across all treatment arms, regardless of subtype. I-SPY2 shows substantially lower estimated EFS hazards for patients achieving pCR, compared to the 5 yr EFS hazard ratio for pCR vs not in Cortazar (hazard ratio 0.49), demonstrating important differences between a metaanalysis compared to a platform trial with uniform high-risk eligibility, standardized pathology assessment, and multiple targeted therapies. Our data support the use of pCR as a primary endpoint for accelerated approval of new drugs if EFS is evaluated in the same population. Based on these findings, the I-SPY2 TRIAL will test whether therapy can be deescalated or escalated for individual patients with the goal of achieving pCR for all.
Title: Pooled analysis of five randomized trials investigating temporary ovarian suppression with gonadotropin-releasing hormone analogs during chemotherapy as a strategy to preserve ovarian function and fertility in premenopausal early breast cancer patients


1 Institut Jules Bordet, Brussels, Belgium; 2 Cleveland Clinic Foundation, Cleveland, OH; 3 Imperial College, London, United Kingdom; 4 GBG - German Breast Group, Neu-Isenburg, Germany; 5 UCSF - University of California, San Francisco, CA; 6 IRCCS AOU San Martino-IST, Genova, Italy; 7 AOU Careggi and Istituto Toscano Tumori, Florence, Italy; 8 SWOG - Fred Hutchinson Cancer Research Center, Seattle, WA; 9 University of Edinburgh, Edinburgh, United Kingdom; 10 Moffitt Cancer Center, Tampa, FL; 11 Loyola University Medical Center, Cardinal Bernardin Cancer Center, Maywood, IL; 12 Tayside Cancer Centre, Ninewells Hospital, Dundee, United Kingdom; 13 University Hospital Rostock, Rostock, Germany; 14 Nexgen Oncology, Dallas, TX; 15 Singleton Hospital, Swansea, United Kingdom and 16 Dana-Farber Cancer Institute, Boston, MA.

Body: Background

The role of temporary ovarian suppression with gonadotropin-releasing hormone analogs (GnRHa) during chemotherapy as a strategy to preserve ovarian function and fertility in premenopausal early breast cancer patients remains highly controversial. This option is considered experimental by the ASCO and ESMO guidelines on fertility preservation in cancer patients. The present pooled analysis aimed at elucidating the efficacy and safety of temporary ovarian suppression with GnRHa during chemotherapy as a strategy to preserve ovarian function and fertility in premenopausal early breast cancer patients.

Patients and methods

This study included individual patient data from 5 trials (PROMISE-GIM6, POEMS/SWOG S0230, Anglo Celtic Group OPTION, GBG-37 ZORO, Moffitt-led trial) in which premenopausal women with early breast cancer were randomized to receive (neo)adjuvant chemotherapy alone or with concurrent administration of GnRHa. Efficacy endpoints were premature ovarian insufficiency (POI, according to the definition used as primary endpoint in the included trials), 1- and 2-year amenorrhea rates and post-treatment pregnancy rate. Safety endpoints were disease-free survival (DFS) and overall survival (OS). Odds ratio (OR), incidence rate ratio (IRR) and hazard ratio (HR) with 95% confidence intervals (CI) were calculated for the effect of adding GnRHa to chemotherapy alone. As each study represents a cluster, statistical analysis has been performed using a random effects model.

The study is registered with the PROSPERO registration number CRD42014015638.

Results

A total of 873 patients from 5 randomized trials were included. Median age was 38 years (interquartile range: 34-42 years). POI rate was 14.1% in the GnRHa group and 30.9% in the control group (adjusted OR 0.38; 95% CI 0.26-0.57; p < 0.001). The incidence of 1-year amenorrhea was 36.8% in the GnRHa group and 40.4% in the control group (adjusted OR 0.92; 95% CI 0.66-1.28; p = 0.623). The incidence of 2-year amenorrhea was 18.2% in the GnRHa group and 30.0% in the control group (adjusted OR 0.51; 95% CI 0.31-0.85; p = 0.009). A total of 37 patients had at least one post-treatment pregnancy in the GnRHa group and 20 in the control group (IRR 1.83; 95% CI 1.06-3.15; p = 0.030).

There were no significant differences in DFS (adjusted HR 1.01; 95% CI 0.72-1.42; p = 0.999) or OS (adjusted HR 0.67; 95% CI 0.42-1.06; p = 0.083) between the GnRHa and control groups.

Subgroup analyses of both efficacy and safety endpoints according to age of the patients, hormone receptor status, type and duration of chemotherapy will be presented at the conference.

Conclusions

This study provides level 1A of evidence for the efficacy and safety of temporary ovarian suppression with GnRHa during chemotherapy in premenopausal early breast cancer patients. Given the findings of this pooled analysis, temporary ovarian suppression with GnRHa during chemotherapy should be considered as a new standard option to reduce the likelihood of chemotherapy-induced POF and possibly improve future fertility in premenopausal early breast cancer patients.
Title: Randomized comparison of adjuvant aromatase inhibitor exemestane (E) plus ovarian function suppression (OFS) vs tamoxifen (T) plus OFS in premenopausal women with hormone receptor positive (HR+) early breast cancer (BC): Update of the combined TEXT and SOFT trials

Body: Background: The combined results of TEXT and SOFT, after 5.7 years median follow-up, found adjuvant E+OFS significantly improved disease-free survival (DFS) vs T+OFS in premenopausal women with HR+ BC (Pagani et al, NEJM 2014). Follow-up was immature for overall survival (OS). We report a planned update with visit cut-off of 31Dec16 after 9 years median follow-up.

Methods: TEXT and SOFT enrolled premenopausal women with HR+ early BC from Nov 2003 to Apr 2011 (2660 TEXT, 3047 SOFT in the intention-to-treat populations). TEXT randomized women within 12wk of surgery to 5 yrs E+OFS vs T+OFS; chemotherapy (CT) was optional and concurrent with OFS. SOFT randomized women to 5 yrs E+OFS vs T+OFS vs T alone, within 12wk of surgery if no CT planned, or within 8mo of completing (neo)adjuvant CT after premenopausal status was (re-)established. OFS was by choice of 5yr GnRH agonist triptorelin, oophorectomy or ovarian irradiation. Both trials were stratified by CT use. The primary endpoint was DFS: randomization until invasive local, regional, distant recurrence or contralateral breast; invasive second malignancy; death. Secondary endpoints included invasive breast cancer-free interval (BCFI), distant recurrence-free interval (DRFI) and OS. Stratified Cox models estimated hazard ratios; Kaplan-Meier method estimated 8yr endpoint rates. NCT00066703/NCT00066690.

Results: DFS for patients assigned E+OFS (n=2346) continued to be significantly improved over T+OFS (n=2344): 8yr DFS was 86.8% vs. 82.8%. The 8yr BCFI was improved by 4.1% (89.3% vs 85.2%) and 8yr DRFI by 2.1% (91.8% vs 89.7%). There was no difference in OS in patients assigned E+OFS vs T+OFS: 93.4% vs 93.3% OS at 8yrs. For 1996 women without CT there have been 45 deaths, with 98% OS at 8yrs with both treatments.

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>N. Events</th>
<th>Hazard Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFS</td>
<td>720</td>
<td>0.77 (0.67-0.90); P&lt;0.001</td>
</tr>
<tr>
<td>BCFI</td>
<td>600</td>
<td>0.74 (0.63-0.87)</td>
</tr>
<tr>
<td>DRFI</td>
<td>433</td>
<td>0.80 (0.65-0.96)</td>
</tr>
<tr>
<td>OS</td>
<td>320</td>
<td>0.98 (0.79-1.22)</td>
</tr>
</tbody>
</table>

Overall toxicity was not significantly worse with E+OFS than with T+OFS (32% vs 31% grade 3-4 targeted AEs). Hot flashes, musculoskeletal symptoms and hypertension were the most frequent targeted grade 3-4 AEs. Overall, 15% of patients stopped all protocol-assigned treatment early. Patients assigned E+OFS had increased risk of assigned oral endocrine therapy cessation (25% vs 19% for patients assigned T+OFS by 4yrs) but not of triptorelin cessation (18% vs 19% by 4yrs, respectively).

Conclusions: After 9 yrs median follow-up, adjuvant E+OFS, as compared with T+OFS, shows a sustained reduction of the risk of recurrence but did not improve overall survival. As in postmenopausal women, oncologists need to consider potential absolute benefits and properly select patients at sufficient risk for recurrence for whom E+OFS seems indicated. Follow-up continues, which will further clarify the effect of E+OFS for safety, late recurrence and overall survival.
Title: Randomized comparison of adjuvant tamoxifen (T) plus ovarian function suppression (OFS) versus tamoxifen in premenopausal women with hormone receptor-positive (HR+) early breast cancer (BC): Update of the SOFT trial

Body: Background: The primary results of SOFT at 5.6 years median follow-up found adding OFS to T did not provide a significant benefit in the overall study population of premenopausal women with HR+ BC (Francis et al, NEJM 2015). For those women at sufficient risk for recurrence to warrant adjuvant chemotherapy (CT) and who remained premenopausal, the addition of OFS improved disease outcomes. Follow-up was immature for overall survival (OS). We report a planned update with visit cut-off of 31Dec16 after 8 yrs median follow-up.

Methods: SOFT randomized premenopausal women with HR+ BC from Nov 2003 to Jan 2011 to 5 yrs of T vs T+OFS vs Exemestane(E)+OFS. OFS was by choice of GnRH agonist triptorelin, oophorectomy or ovarian irradiation. SOFT was stratified by the use of prior CT; 47% received no CT and 53% remained premenopausal after prior CT, determined by premenopausal estradiol level within 8 months of CT completion. The primary endpoint was invasive disease-free survival (DFS; randomization until invasive local, regional, distant recurrence or contralateral breast; invasive second malignancy; death). Secondary endpoints included invasive breast cancer-free interval (BCFI), distant recurrence-free interval (DRFI) and OS. NCT00066690.

Results: DFS for patients assigned T+OFS (n=1015) was significantly improved over T (n=1018; HR=0.76 [95%CI 0.62-0.93]) and 8yr DFS was 83.2% vs 78.9%, respectively; BCFI and DRFI results were supportive (see Table). Hazard ratios for these 3 endpoints showed no heterogeneity by use of prior CT. For patients with prior CT, 8yr DFS was 76.7% with T+OFS vs 71.4% with T (Δ=5.3%); in those without CT, 8yr DFS was 90.6% vs 87.4% (Δ=3.2%). E+OFS (n=1014) improved outcomes relative to T (Table); 8yr DFS for E+OFS was 85.9% (80.4% with use of prior CT and 92.5% for those without CT). OS was improved with T+OFS vs T (8yr OS 93.3% vs 91.5%). 8yr OS was 92.1% with E+OFS. 201/225 deaths occurred in women with prior CT. For women without CT there have been 10, 5 and 9 deaths in the T+OFS, T and E+OFS groups (total n=1419), respectively, only half of these deaths after breast cancer event.

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>N. Events</th>
<th>Hazard Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFS</td>
<td>518</td>
<td>0.76 (0.62-0.93) P=0.009</td>
</tr>
<tr>
<td>BCFI</td>
<td>437</td>
<td>0.76 (0.61-0.95)</td>
</tr>
<tr>
<td>DRFI</td>
<td>306</td>
<td>0.86 (0.66-1.13)</td>
</tr>
<tr>
<td>OS</td>
<td>225</td>
<td>0.67 (0.48-0.92)</td>
</tr>
</tbody>
</table>

Overall toxicity was worse with T+ OFS than with T, including 32% vs 25% grade 3+ targeted AEs. Early cessation of tamoxifen occurred for 19% assigned T+OFS and 22% of women assigned T; the cumulative incidence of early cessation of triptorelin on the T+OFS arm was 23% by 4yrs. Early cessation of exemestane occurred for 28% and of triptorelin for 21% by 4yrs on the E+OFS arm.

Conclusions: With additional follow-up to a median of 8yrs, SOFT further supports the value of OFS for some premenopausal women. Follow-up continues, which will further clarify the safety and the benefit of OFS for late recurrence and overall survival. Oncologists appear to be able to select a low risk group (no chemotherapy) for whom treatment escalation is unlikely to improve survival.
Title: Randomized blinded sham- and waitlist-controlled trial of acupuncture for joint symptoms related to aromatase inhibitors in women with early stage breast cancer (S1200)

Dawn L Hershman¹, Joseph M Unger², Heather Greenlee³, Jillian Capodice³, Danika L Lew², Alice T Kengla⁴, Marianne K Melnik⁵, Carla W Jorgensen⁶, William H Kreisle⁷, Lori M Minasian⁸, Michael J Fisch⁹, Lynn Henry¹⁰ and Katherine D Crew¹. ¹Columbia University Medical Center, New York, NY; ²Fred Hutchinson Cancer Research Center, Seattle, WA; ³Mount Sinai Hospital, New York, NY; ⁴Kaiser Permanente Medical Center, Walnut Creek, CA; ⁵Spectrum Health Medical Group, Grand Rapids, MI; ⁶NCORP of the Carolinas (Greenville Health System), Greenville, SC; ⁷St. Luke’s Mountain States Tumor Institute, Boise, ID; ⁸National Cancer Institute, Bethesda, MD; ⁹AIM Specialty Health, Chicago, IL and ¹⁰University of Utah Huntsman Cancer Institute, Salt Lake City, UT.

Body: Background: Musculoskeletal symptoms are the most common side effect of aromatase inhibitors (AIs) and can result in decreased quality of life and discontinuation of therapy. Pilot data from two prior single institution studies showed that acupuncture decreased AI-induced joint symptoms in breast cancer (BC) patients.

Methods: We conducted a SWOG multicenter randomized controlled trial among postmenopausal women with early stage BC. Patients taking an AI for ≥30 days and having a worst pain score of ≥3 out of 10 using the Brief Pain Inventory (BPI-WP) were eligible. Subjects were randomized at a 2:1:1 ratio to true acupuncture (TA) vs. sham acupuncture (SA) vs. waitlist control (WC).

The TA protocol used a standardized protocol of body and auricular acupoints tailored to joint symptoms. The similarly standardized SA protocol utilized superficial needling of non-acupoints. Both the TA and SA protocols consisted of a 12 week intervention, with 12 sessions administered over 6 weeks, followed by 1 session per week for 6 additional weeks. The primary endpoint was change in the BPI-WP (worst pain) score at 6 weeks. Secondary outcomes included other BPI scores, the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) for the hips and knees, the Modified Score for the Assessment of Chronic Rheumatoid Affections of the Hands (M-SACRAH), and functional testing with grip strength and “Timed Get Up and Go” (TGUG). The design specified alpha=.025 two-sided tests to account for two independent comparisons (TA vs. SA and TA vs. WC).

Results: Among 226 patients registered, 110 were randomized to TA, 59 to SA and 57 to WC. Baseline characteristics were similar between the groups. In a linear regression adjusting for the baseline score and stratification factors, 6-week mean BPI-WP scores were 0.92 points lower (correlating with less pain) in the TA compared to SA arm (95% CI: 0.20-1.65, p=.01), and were 0.96 points lower in the TA compared to WC arm (95% CI: 0.24-1.67, p=.01). The proportion of patients experiencing a clinically meaningful (>2) reduction (i.e. improvement) in BPI-WP was 58% for TA compared to 33% on SA and 31% on WC. Patients randomized to TA had improved symptoms compared to SA at week 6 according to all other BPI pain measures (average pain, p=.04; pain interference, p=.02; pain severity, p=.05; worst stiffness, p=.02). Results were similar compared to WC. Patients randomized to TA compared to SA or WC had statistically significant or marginally statistically significant improvements in BPI pain measures at week 12. Patients randomized to TA had generally improved symptoms compared to SA at week 6 according to all other BPI pain measures (average pain, p=.04; pain interference, p=.02; pain severity, p=.05; worst stiffness, p=.02). Results were similar compared to WC. Patients randomized to TA compared to SA or WC had statistically significant or marginally statistically significant improvements in BPI pain measures at week 12. Patients randomized to TA had generally improved symptoms compared to SA at week 6 and at week 12 according to the M-SACRAH and WOMAC measures (p<0.05). With regard to adverse events, more patients on the TA arm experienced Grade 1 bruising compared to SA (47% vs. 25%, p=.01). No other differences by arm for selected adverse events were observed.

Conclusions: This study was the first large multicenter trial to investigate the effect of acupuncture in treating AI-induced joint symptoms in BC patients. According to multiple measures, TA generated better outcomes than either SA or WC with minimal toxicity.
Cancer risks and response to targeted therapy associated with BRCA2 variants of uncertain significance

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¹Mayo Clinic, Rochester, MN and ²Duke University, Durham, NC.

Background: Germline genetic testing of individuals with a diagnosis of triple negative breast cancer, young age at diagnosis, or a family history of breast and/or ovarian cancer has led to the identification of many unique BRCA2 missense variants of uncertain significance (VUS). VUS in BRCA2 are predominantly missense mutations that have unclear relevance to breast, ovarian, and other cancers. Thus, patients found to have a germline BRCA2 VUS do not know if the variant is associated with high risks of these cancers similar to truncating mutations, intermediate risks more similar to CHEK2 mutations, or low risks of no clinical significance. Furthermore, it is unclear if germline BRCA2 VUS, or somatic VUS identified by tumor sequencing, are associated with hypersensitivity to selected DNA damaging and cross-linking agents.

Methods: We have used a homology directed repair (HDR) cell-based assay to characterize missense variants in the DNA binding domain (DBD) of BRCA2. The method has been validated using known pathogenic and known non-pathogenic BRCA2 missense variants and has 100% sensitivity (95% confidence interval (CI): 75.3%–100%) and 100% specificity (95% CI: 81.5%–100%) for pathogenic BRCA2 variants. A classifier of variant pathogenicity based on the mean and variances of the HDR results of the known pathogenic and neutral variants has been established. We have also developed PARP inhibitor and cisplatin drug response assays for BRCA2 missense variants.

Results: Assessment of 207 BRCA2 missense variants, identified in public databases such as BRCA exchange and ClinVar, by the HDR assay identified 71 deleterious variants with >99% probability of pathogenicity, 116 neutral variants with >99% probability of neutrality, and 20 with hypomorphic activity and potentially intermediate risk. A combination of the functional data and sequence-based predictors of protein activity in a Bayesian prediction model resulted in classification of the deleterious variants as pathogenic cancer predisposing variants and the neutral variants as non-pathogenic with low clinical significance. The influence of the deleterious/pathogenic variants on PARPi and cisplatin response was also assessed.

Conclusion: The HDR assay is effective for characterization of BRCA2 VUS. The combination of functional data and in silico prediction models provides a robust tool for clinical annotation of BRCA2 VUS. HDR function of BRCA2 missense variants is strongly correlated with response to targeted therapy.
Title: Results from a randomized placebo-controlled phase 2 trial evaluating exemestane ± enzalutamide in patients with hormone receptor–positive breast cancer

Ian Krop¹, Vandana Abramson², Marco Colleoni³, Tiffany Traina⁴, Frankie Holmes⁵, Laura Estevez⁶, Lowell Hart⁷, Ahmad Awada⁸, Claudio Zamagni⁹, Patrick Morris¹⁰, Lee Schwartzberg¹¹, Stephen Chan¹², Duncan Wheatley¹³, Ayca Gucalp¹⁴, Laura Biganzoli¹⁵, Joyce Steinberg¹⁶, Luca Giannini¹⁷, Maureen Trudeau¹⁸, Iulia Cristina Tudor¹⁹, Denka Markova¹⁹, Elly Barry¹⁰, Jamal Tarazi¹⁰, Ross Stewart¹⁰, Eric Winer¹ and Denise A Yardley²¹,²².

¹Dana-Farber Cancer Institute, Boston, MA; ²Vanderbilt University, Nashville, TN; ³Istituto Europeo di Oncologia, Milan, Italy; ⁴Memorial Sloan-Kettering Cancer Center, New York, NY; ⁵Texas Oncology-Houston Memorial City, Houston, TX; ⁶Centro Integral Oncológico Clara Campal, Hospital de Madrid Norte-Sanchinarro, Madrid, Spain; ⁷Florida Cancer Specialists, Fort Myers, FL; ⁸Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium; ⁹Azienda Ospedaliera Universitaria di Bologna Policlinico S. Orsola Malpighi, Bologna, Italy; ¹⁰Beaumont Hospital, Dublin, Ireland; ¹¹University of Tennessee, West Cancer Center, Memphis, TN; ¹²Nottingham University Hospital, City Campus, Nottingham, United Kingdom; ¹³Royal Cornwall Hospitals NHS Trust-Sunrise Centre, Cornwall, United Kingdom; ¹⁴Weil Cornell College of Medicine, New York, NY; ¹⁵Nuovo Ospedale di Prato, Italy; ¹⁶Astellas Pharma Inc, Northbrook, IL; ¹⁷Ospedale San Raffaele, Milan, Italy; ¹⁸Sunnybrook Health Sciences Centre, Toronto, ON, Canada; ¹⁹Medivation, Inc. (Medivation, Inc., was Acquired by Pfizer, Inc., in September 2016), San Francisco, CA; ²⁰Pfizer, Inc., New York, NY; ²¹Sarah Cannon Research Institute, Nashville, TN and ²²Tennessee Oncology, PLLC, Nashville, TN.

Body: Background: The androgen receptor (AR) is expressed in >75% of hormone receptor (HR)+ tumors. AR signaling has been associated with resistance to endocrine therapy (ET). Aromatase inhibitors (AIs) divert estrogen precursors to androgens; in preclinical models enzalutamide (ENZA) blocked both estrogen- and androgen-mediated growth of HR+ cells. In a phase 1 study of ET+ENZA in breast cancer, doubling the dose of exemestane (EXE) to 50 mg was necessary to restore exposure observed with 25 mg.¹

Methods: This placebo (PBO)–controlled phase 2 trial randomized patients (pts) with HR+/HER2-normal advanced/metastatic breast cancer (MBC) to either 25 mg EXE+PBO or 50 mg EXE+160 mg ENZA daily (NCT02007512). Two parallel cohorts enrolled pts who had no prior ET (C1) or who had received 1 prior ET for MBC (C2). Randomization was stratified on resistance to prior ET and prior exposure to AI. Tissue samples for biomarker development were mandatory. Brain metastases or a history of seizure was exclusionary. One prior chemotherapy regimen for MBC was permitted. Response was assessed every 8 weeks for 48 weeks, then every 12 weeks. Crossover to ENZA+EXE was allowed at disease progression. A gene signature–based biomarker (Bmkr) indicating AR signaling predictive of response to ENZA was developed using a training set of RNAseq data from 2/3 of randomized pts and validated using a test set of data from the remaining 1/3 of pts. Progression-free survival (PFS) according to RECIST v1.1 was the primary endpoint in the intent-to-treat (ITT) population and in the Bmkr+ subgroup of each cohort. Secondary endpoints included clinical benefit rate at 24 weeks (CBR24), best overall response, and safety.

Results: A total of 247 pts were randomized (C1, n=127; C2, n=120). In C1, 50 pts (39.4%) were Bmkr+; in C2, 53 pts (27.8%) were Bmkr+. Statistically significant improvements in median PFS and CBR24 were observed only in the Bmkr+ population with no prior ET (Table). The most common adverse events (AEs) reported in the ENZA+EXE arms were nausea (39%) in C1 and fatigue (37%) in C2. In C1, 9 pts (15%) and 10 pts (16%) discontinued the study due to AEs in the ENZA+EXE and PBO+EXE arms, respectively. In C2, 11 pts (18%) and 5 pts (8%) discontinued due to AEs in the ENZA+EXE and PBO+EXE arms, respectively.

Conclusions: In the first reported randomized trial of ENZA in HR+ MBC, ENZA+EXE was well tolerated with no new safety signals. The study met its primary endpoint in pts with Bmkr+ MBC with no prior ET.


<table>
<thead>
<tr>
<th>C1: No Prior ET</th>
<th>C2: 1 Prior ET</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITT</td>
<td>Bmkr+</td>
</tr>
<tr>
<td>ITT</td>
<td>Bmkr+</td>
</tr>
<tr>
<td></td>
<td>EXE+</td>
</tr>
<tr>
<td>----------------</td>
<td>------</td>
</tr>
<tr>
<td></td>
<td>n=63</td>
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<td>Primary endpoint</td>
<td></td>
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<tr>
<td>PFS, median, months</td>
<td>11.8</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(7.3, 15.9)</td>
</tr>
<tr>
<td>Hazard ratio</td>
<td>0.82</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(0.54, 1.26)</td>
</tr>
<tr>
<td>P value</td>
<td>0.3631</td>
</tr>
<tr>
<td>Secondary endpoint</td>
<td></td>
</tr>
<tr>
<td>CBR24, n (%)</td>
<td>39 (62)</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(49, 74)</td>
</tr>
<tr>
<td>P value</td>
<td>0.0609</td>
</tr>
</tbody>
</table>
Title: Appropriate margins for breast conserving surgery in patients with early stage breast cancer: A meta-analysis

Chirag Shah¹, Vivek Verma², Harlan Sayles², Abram Recht³ and Frank Vicini⁴. ¹Cleveland Clinic, Cleveland, OH; ²University of Nebraska Medical Center, Omaha, NE; ³Beth Israel Deaconess Medical Center/Harvard Medical School, Boston, MA and ⁴21st Century Oncology/Michigan Healthcare Professionals, Farmington Hills, MI.

Body: Background: Significant controversy exists regarding the appropriate minimum tumor-free margin width for patients undergoing breast-conserving therapy. The SSO-ASTRO guidelines recommend 'no ink on tumor' based upon a meta-analysis of 33 studies, but this had significant limitations. In order to address these concerns, a meta-analysis of all available data (using 31 of the initial studies) was performed but employing stricter criteria for acceptability and quality of evaluable studies.

Methods: Study eligibility criteria were: (1) minimum follow-up of 50 months; (2) explicit pathologic criteria for defining margins; and (3) consistent endpoints associated with local recurrence. The compiled studies were analyzed using generalized linear mixed models for the outcome of local recurrence, with random effects for study and fixed effects for various patient and study characteristics.

Results: The analysis incorporated 38 studies, which included 55,302 patients treated from 1968-2010. Two of the previous studies were excluded (one because of short follow-up, and the second because it was updated) with seven new studies added from the previous meta-analysis including an update of a previously included analysis. Median follow-up was 7.2 years. The median age of the cohort was 55 years, 74% of patients had T1 tumors, and 72% were node negative. The crude rate of local recurrence for patients with positive margins was 10.3%, compared to 3.8% for those with negative margins defined as no tumor on ink or wider (p<0.001). The crude rates of local recurrence decreased as the margin distance increased: 7.2% for patients with margins >0-<2 mm, 3.6% for margins of 2-5 mm (3.6%), and 3.2% for margins wider than 5 mm (p<0.001 for each). Use of endocrine therapy and increasing median study year were associated with a reduction in local recurrence in univariate models but not in multivariable analyses.

Conclusions: The current meta-analysis indicates that having margin widths 2 mm or greater is associated with a lower risk of ipsilateral breast failure than narrower but uninvolved margins. It therefore fails to confirm that 'no-tumor on ink' is optimal. Further analyses are needed to clarify this issue, particularly to identify the critical minimum tumor-free margin for different patient subgroups.
2017 San Antonio Breast Cancer Symposium

Publication Number: GS5-02

Title: Axillary dissection vs. no axillary dissection in patients with cT1-T2cN0M0 breast cancer and only micrometastases in the sentinel node(s): Ten-year results of the IBCSG 23-01 trial

Viviana Galimberti1, Bernard F Cole1, Giuseppe Viale1, Paolo Veronesi1, Elisa Vicini1, Mattia Intra1, Giovanni Mazzarol1, Samuele Massarut1, Janez Zgajnar1, Mario Taffurelli1, David Littlejohn1, Tina Egli1, Carlo Tondini1, Angelo Di Leo1, Marco Colleoni1, Meredith M Regan1, Alan S Coates1, Richard D Gelber1 and Aron Goldhirsch1. 1International Breast Cancer Study Group Trial 23-01 Investigators.

Body: BACKGROUND: The phase III IBCSG 23-01 multicenter, randomized, non-inferiority trial compared disease-free survival (DFS) in breast cancer patients with one or more micrometastatic (≤2 mm) sentinel nodes (SNs) randomized to either axillary dissection (AD) or no axillary dissection (no-AD). Results after 5 years showed no difference in DFS between the arms. Here we report results after a median follow-up of 9.8 years.

METHODS: Eligible patients had cancers of pathological diameter ≤5 cm and one or more micrometastatic (≤2 mm) foci, including isolated tumor cells, in the SNs. Patients with axillary macrometastases were excluded. Breast surgery was conservative or mastectomy. Eligible patients were randomized to AD vs. no-AD. The primary endpoint was disease-free survival (DFS); secondary endpoints were overall survival (OS), site of recurrence (particularly axillary recurrence), and surgical complications of AD. DFS and OS were estimated using the product-limit method, and the log-rank test was used to compare the treatment groups. Patients without a DFS or OS event were censored at the date of last follow-up. Non-inferiority margin for no-AD vs. AD was defined as a DFS hazard ratio (HR, no-AD relative to AD) of <1.25, and was assessed using a z-test applied to the log HR. Active follow-up of patients was terminated in February 2017.

RESULTS: From 2001 to 2010, 934 patients were randomized at 27 centers; 931 were evaluable (467 in the no-AD group and 464 in the AD group). Median follow-up was 9.8 (IQR: 7.8–12.7) years. The number and types of first DFS events according to treatment group are shown in the Table.

<table>
<thead>
<tr>
<th>Disease-free Survival Events</th>
<th>No-AD</th>
<th>AD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td>101</td>
<td>117</td>
</tr>
<tr>
<td><strong>Breast cancer related events</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Local</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>Contralateral breast</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>Distant</td>
<td>41</td>
<td>47</td>
</tr>
<tr>
<td><strong>Non-breast cancer related events</strong></td>
<td>27</td>
<td>42</td>
</tr>
<tr>
<td>Second malignancies</td>
<td>17</td>
<td>23</td>
</tr>
<tr>
<td>Death without prior cancer event</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Death with unknown cancer status</td>
<td>4</td>
<td>17</td>
</tr>
</tbody>
</table>

10-year DFS was 75% (95% confidence interval [CI]: 72%–81%) in the no-AD group and 75% (95% CI: 71%–79%) in the AD group (HR [no-AD vs. AD]=0.85; 95% CI: 0.65–1.11; log-rank p=0.23; non-inferiority p=0.002). There were 45 deaths in the no-AD group and 58 in the AD group. 10-year OS was 91% (95% CI: 88%–94%) in the no-AD group and 88% (95% CI: 85%–92%) in the AD group (HR [no-AD vs. AD]=0.77; 95% CI: 0.56–1.07; log-rank p=0.19).

CONCLUSION: Findings after a median follow-up of 9.8 years fully support the findings at 5 years in that no-AD is not inferior to AD with respect to DFS, and there is no significant difference between the arms for DFS and OS, thus confirming that AD is not
indicated in patients with micrometastatic SNs.
2017 San Antonio Breast Cancer Symposium

Publication Number: GS5-03

Title: Risk of arm morbidity after local therapy in young breast cancer survivors

A Kuijer\textsuperscript{1,2,3,4}, L S Dominici\textsuperscript{1,2,3}, S M Rosenberg\textsuperscript{1,3}, J Hu\textsuperscript{1}, S Gelber\textsuperscript{1}, S Di Lascio\textsuperscript{1}, K J Ruddy\textsuperscript{5}, J Wong\textsuperscript{1,2,3}, R M Tamimi\textsuperscript{2,3}, L Schapira\textsuperscript{6}, V F Borges\textsuperscript{7}, S E Come\textsuperscript{3,8}, K Sprunck-Harrild\textsuperscript{1}, A H Partridge\textsuperscript{1,3} and T A King\textsuperscript{1,2,3}.\textsuperscript{1}Dana-Farber Cancer Institute, Boston, MA;\textsuperscript{2}Brigham and Women's Hospital, Boston, MA;\textsuperscript{3}Harvard Medical School, Boston, MA;\textsuperscript{4}Diakonessenhuis Utrecht, Utrecht, Netherlands;\textsuperscript{5}Massachusetts General Hospital, Boston, MA;\textsuperscript{6}Stanford Cancer Institute and/or Stanford University, Stanford, CA;\textsuperscript{7}University of Colorado Cancer Center, Aurora, CO;\textsuperscript{8}Beth Israel Deaconess, Boston, MA and \textsuperscript{9}Mayo Clinic, Rochester, MN.

Body: BACKGROUND: Arm morbidity, the most reported comorbidity following axillary surgery for breast cancer, is of particular importance in young patients given the longer survivorship period and detrimental effects of arm-morbidity on body image and social-, home- and personal care functions. We assessed the incidence of arm-morbidity stratified by local therapy strategies in young women enrolled in the Young Women's Breast Cancer Study (YWS).

PATIENTS AND METHODS: The YWS, a multicenter prospective cohort study, established to explore biological, medical and psychosocial issues in young breast cancer patients, enrolled 1302 women with stage 0-4 breast cancer ≤40 years of age from October 2006 to June 2016. For this analysis, we examined incidence of patient reported arm-swelling or decreased range of motion (ROM) 1-year after diagnosis using relevant items of the CARES-SF. Patients with stage 4 disease (n = 60), those for whom no information on arm-morbidity was available (n=198) and those with bilateral cancer with different local therapy strategies on each side (n=7), were excluded. We performed logistic regression analyses to identify risk factors for arm morbidity.

RESULTS: Among 1037 patients (median age 37 years), 13% and 40% reported arm-swelling or decreased ROM, respectively, in the ipsilateral arm at 1-year. 52% (n=539) of patients underwent SLNB and 39% (n=407) ALND. The incidence of arm-swelling was 4% (11/280) in patients who underwent SLNB without RT, 8% (21/252) in patients who underwent SLNB with RT, 20% (13/66) in patients who underwent ALND without RT and 24% (84/337) in patients who received ALND with RT. The incidence of decreased ROM was 21% (59) in patients who underwent SLNB without RT, 34% (86) in patients who underwent SLNB with RT, 33% (22) in patients who underwent ALND without RT and 44% (148) in patients who received ALND with RT. Being overweight, uncomfortable financial status, T4 tumors, ALND and RT were independently associated with an increased risk of arm-swelling. Overweight, mastectomy and RT were independently associated with an increased risk of a decreased ROM.

<table>
<thead>
<tr>
<th></th>
<th>Arm swelling at 1 year (n=137)</th>
<th>Decreased ROM at 1 year (n=335)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>p</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;18.5</td>
<td>0.7</td>
<td>0.62</td>
</tr>
<tr>
<td>18.5-24.9</td>
<td>ref</td>
<td></td>
</tr>
<tr>
<td>25-29.9</td>
<td>1.7</td>
<td>0.03</td>
</tr>
<tr>
<td>≥30</td>
<td>1.1</td>
<td>0.79</td>
</tr>
<tr>
<td>Financial comfort</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comfortable</td>
<td>ref</td>
<td></td>
</tr>
<tr>
<td>Uncomfortable</td>
<td>0.6</td>
<td>0.02</td>
</tr>
<tr>
<td>pT stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pT1</td>
<td>ref</td>
<td></td>
</tr>
<tr>
<td>pT2</td>
<td>1.2</td>
<td>0.62</td>
</tr>
<tr>
<td>pT3</td>
<td>1.2</td>
<td>0.70</td>
</tr>
<tr>
<td>pT4</td>
<td>4.4</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Table 1). Results of the logistic regression analyses.
<table>
<thead>
<tr>
<th>pN0</th>
<th>ref</th>
<th></th>
<th>ref</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>pN1</td>
<td>1.1</td>
<td>0.85</td>
<td>1.7</td>
<td>0.08</td>
</tr>
<tr>
<td>pN2</td>
<td>3.0</td>
<td>0.11</td>
<td>1.9</td>
<td>0.27</td>
</tr>
<tr>
<td>pN3</td>
<td>3.2</td>
<td>0.11</td>
<td>2.7</td>
<td>0.16</td>
</tr>
<tr>
<td>Surgery</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCS</td>
<td>ref</td>
<td></td>
<td>ref</td>
<td></td>
</tr>
<tr>
<td>Mastectomy</td>
<td>1.2</td>
<td>0.61</td>
<td>1.8</td>
<td>0.02</td>
</tr>
<tr>
<td>Bilateral mastectomy</td>
<td>1.1</td>
<td>0.84</td>
<td>1.6</td>
<td>0.06</td>
</tr>
<tr>
<td>Axillary surgery</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SLNB</td>
<td>ref</td>
<td></td>
<td>ref</td>
<td></td>
</tr>
<tr>
<td>SLNB + ALND</td>
<td>3</td>
<td>&lt;0.01</td>
<td>1.3</td>
<td>0.33</td>
</tr>
<tr>
<td>ALND</td>
<td>3.6</td>
<td>&lt;0.01</td>
<td>1.0</td>
<td>0.90</td>
</tr>
<tr>
<td>RT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>ref</td>
<td></td>
<td>ref</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1.8</td>
<td>0.05</td>
<td>2.4</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Note. In these analysis we also corrected for age, incidence year, employment status, stage of disease, reconstructive surgery, and chemotherapy treatment.

**CONCLUSION:** Patient reported outcomes reveal high rates of arm-swelling and decreased ROM 1 year after breast cancer diagnosis in a large prospective cohort of young breast cancer survivors. These findings suggest an opportunity for pre-operative education and early intervention for arm impairment in this population.
2017 San Antonio Breast Cancer Symposium

Publication Number: GS5-04

Title: Prediction of occult invasive disease in ductal carcinoma in situ using deep learning features

Joseph Y Lo¹, Lars J Grimm¹, Maciej A Mazurowski¹, Jay A Baker¹, Jeffrey R Marks², Lorraine M King², Carlo C Maley³, Eun-Sil Shelley Hwang² and Bibo Shi¹. ¹Carl E. Ravin Advanced Imaging Laboratories, Duke University School of Medicine, Durham, NC; ²Duke University School of Medicine, Durham, NC and ³Biodesign Center for Personalized Diagnostics and School of Life Sciences, Arizona State University, Tempe, AZ.

Body: Background: Deep learning, especially deep convolutional neural network (CNN), has emerged as a promising approach for many image recognition or classification tasks, demonstrating human or even superhuman performance. Used as feature extractor, some pre-trained CNN models can match or surpass the performance of domain-specific, “handcrafted” features. In this study, we aim to determine whether deep features extracted from digital mammograms using a pre-trained deep CNN are prognostic of occult invasive disease for patients with ductal carcinoma in situ (DCIS) on core needle biopsy.

Materials and Methods: In this retrospective study, we collected digital mammography magnification views for 99 subjects with DCIS at biopsy, 25 of which were subsequently upstaged to invasive cancer. We utilized a deep CNN model that was pre-trained on non-medical images (e.g., animals, plants, instruments) as the feature extractor. Through a statistical pooling strategy, we extracted deep features at different levels of convolutional layers from the lesion areas, without sacrificing the original resolution or distorting the underlying topology. A multivariate classifier was then trained to predict which tumors contain occult invasive disease. This was compared to the performance of traditional “handcrafted” computer vision (CV) features previously developed specifically to assess mammographic calcifications. The generalization performance was assessed using Monte Carlo cross validation and receiver operating characteristic (ROC) curve analysis.

Results: Deep features were able to distinguish DCIS with occult invasion from pure DCIS, with an area under the ROC curve (AUC-ROC) equal to 0.70 (95% CI: 0.68-0.73). This performance was comparable to the “handcrafted” CV features (AUC-ROC = 0.68, 95% CI: 0.66-0.71) that were designed with prior domain knowledge.

Conclusion: In spite of being pre-trained on only non-medical images, the deep features extracted from digital mammograms demonstrated comparable performance to “handcrafted” CV features for the challenging task of predicting DCIS upstaging.

Acknowledgments: This work was supported in part by NIH/NCI R01-CQA185138 and DOD Breast Cancer Research Program W81XWH-14-1-0473.
Title: Primary endocrine therapy for ER-positive ductal carcinoma in situ (DCIS) CALGB 40903 (Alliance)

E Shelley Hwang\textsuperscript{1}, Stephanie Duong\textsuperscript{2}, Isabelle Bedrosian\textsuperscript{3}, Jacob Allred\textsuperscript{2}, Dorota Wisner\textsuperscript{4}, Terry Hyslop\textsuperscript{1}, Abigail Caudle\textsuperscript{3}, Joesph Guenther\textsuperscript{5}, Cliff Hudis\textsuperscript{6}, Eric Winer\textsuperscript{7}, Laura Esserman\textsuperscript{4} and Nola Hylton\textsuperscript{4}. \textsuperscript{1}Duke University, Durham, NC; \textsuperscript{2}Mayo Clinic, Rochester, MN; \textsuperscript{3}MD Anderson, Houston, TX; \textsuperscript{4}UCSF, San Francisco, CA; \textsuperscript{5}St. Elizabeth Medical Center South, Edgewood, KY; \textsuperscript{6}ASCO, Alexandria, VA and \textsuperscript{7}Dana Farber Cancer Institute, Boston, MA.

Body: Background: Standard treatment for ductal carcinoma in situ (DCIS) consists of surgery, often followed by adjuvant radiation therapy or endocrine therapy. This current approach is thought to represent overtreatment for some patients. This study was undertaken to determine whether an alternate approach of primary endocrine therapy alone could result in measurable radiographic changes in unresected estrogen receptor (ER)-positive DCIS.

Methods: A phase II open-label single arm multi-center cooperative group trial (CALGB 40903) was conducted for postmenopausal patients diagnosed with ER-positive DCIS. All eligible patients underwent baseline mammography and MRI, followed by 6 months of preoperative therapy with letrozole. Follow up breast MRI was obtained at 3 and 6 months of treatment. The primary endpoint was change in total MRI volume of DCIS enhancement from baseline to 3 months and from baseline to 6 months. Secondary endpoints were change in MRI maximum diameter over baseline and change in mammographic extent of disease over baseline. Endpoints were analyzed via 2-sided paired t-tests (a=0.05).

Results: From 8/1/12 to 2/1/16 108 patients were enrolled; Of the 77 patients who completed letrozole treatment per protocol, 66 patients were assessable. 2 additional patients who did not complete treatment per protocol were considered assessable and thus included in the analysis for a total of 68 patients. Median age of the cohort was 62.7 years. DCIS nuclear grade was low in 10% of patients, intermediate in 49%, and high in 40%. 82% of patients had ER-positive, PR-positive DCIS. The total mean MRI volume decreased from baseline to 3 months by 1.93 cm\textsuperscript{3} (p<0.001) and from baseline to 6 months by 1.82 cm\textsuperscript{3} (p<0.001). There was no significant difference in tumor volume between 3 and 6 months. Mean total mammographic tumor diameter decreased from baseline to 6 months by 3.31 mm\textsuperscript{2} (p=0.078).

Conclusions: In a cohort of postmenopausal women treated with 6 months of preoperative endocrine therapy for ER-positive DCIS, MRI volume decreased markedly by 3 months, while mammographic extent of disease was not altered significantly. Correlation of imaging changes with pathology and baseline biomarkers will be conducted. These results will help determine whether MRI could be an effective modality for monitoring treatment response in some patients treated with primary endocrine therapy for ER-positive DCIS.
**Title:** A U.S. food and drug administration pooled analysis of outcomes of older women with hormone-receptor positive metastatic breast cancer treated with a CDK4/6 inhibitor as initial endocrine based therapy

Harpreet Singh1, Lynn J Howie1, Erik Bloomquist1, Suparna Wedam1, Laleh Amiri-Kordestani1, Shengui Tang1, Rajeshwari Sridhara1, Amna Ibrahim1, Kirsten Goldberg1, Amy McKee1, Julia A Beaver1 and Richard Pazdur1. 1US Food and Drug Administration, Silver Spring, MD.

**Body:** Background: With recent FDA approvals of inhibitors of Cyclin Dependent Kinases 4 and 6 (CDK 4/6) in combination with endocrine therapy for the treatment of postmenopausal women with hormone-receptor positive metastatic breast cancer (MBC), an increasing number of older adults will be treated with this class of agents. An improved understanding of the safety and efficacy of CDK 4/6 inhibitors in this population is important to inform clinical decision making for the treatment of older patients.

**Methods:** Data from two prospective randomized controlled studies (n=1334) of different CDK 4/6 inhibitors in combination with an aromatase inhibitor for the initial treatment of postmenopausal patients with hormone-receptor positive MBC were pooled and analyzed. The effect of age on progression free survival (PFS) was explored using Kaplan Meier (KM) estimates and a Cox-proportional hazard model. Safety analysis included adverse events up to 30 days after last administration of drug based on standardized adverse event datasets.

**Results:** Age was balanced between the two studies, and between treatment arms within each study. The median age of women was 62 (range 23-91). Of the 1334 total patients, 42% were ≥65, and 24% were ≥70. For patients ≥70 who were treated with a CDK4/6 inhibitor in combination with an aromatase inhibitor, the estimated PFS was not reached (95% CI: 25.1months, NR) vs an estimated 18 months (95% CI: 13.8, 31.3) for those treated only with an aromatase inhibitor. For patients <70 treated with a CDK4/6 inhibitor, the estimated PFS was 23.5 months (95% CI: 21.4, 25.7) vs an estimated 13.8 months (95% CI: 12.9, 16.5) for those treated only with an aromatase inhibitor.

Safety was evaluated in the 778 patients who received at least one dose of CDK4/6 inhibitor.

**Adverse Events by Age in Patients Treated with a CDK4/6 Inhibitor**

<table>
<thead>
<tr>
<th>Event</th>
<th>Patients &lt; 65 years</th>
<th>Patients ≥65 years</th>
<th>Patients ≥70 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=447</td>
<td>N=331</td>
<td>N=187</td>
</tr>
<tr>
<td>Grade 1-2 Adverse Events</td>
<td>437 (98)</td>
<td>324 (98)</td>
<td>185 (99)</td>
</tr>
<tr>
<td>Grade 3-4 Adverse Events</td>
<td>340 (76)</td>
<td>276 (83)</td>
<td>159 (85)</td>
</tr>
<tr>
<td>Serious Adverse Events</td>
<td>72 (16)</td>
<td>88 (27)</td>
<td>50 (27)</td>
</tr>
<tr>
<td>Adverse Events Leading to Discontinuation</td>
<td>35 (8)</td>
<td>56 (17)</td>
<td>38 (20)</td>
</tr>
<tr>
<td>Adverse Events leading to dose reduction and/or interruption</td>
<td>323 (72)</td>
<td>253 (76)</td>
<td>147 (79)</td>
</tr>
</tbody>
</table>

**Selected Adverse Events**

<table>
<thead>
<tr>
<th>Event</th>
<th>Patients &lt; 65 years</th>
<th>Patients ≥65 years</th>
<th>Patients ≥70 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutropenia (all grades)</td>
<td>341 (76)</td>
<td>256 (77)</td>
<td>150 (80)</td>
</tr>
<tr>
<td>Grade 3-4 neutropenia</td>
<td>292 (65)</td>
<td>228 (69)</td>
<td>134 (72)</td>
</tr>
<tr>
<td>Infections (all grades)</td>
<td>190 (43)</td>
<td>165 (50)</td>
<td>100 (53)</td>
</tr>
<tr>
<td>Hepatotoxicity (all grades)</td>
<td>79 (18)</td>
<td>51 (15)</td>
<td>34 (18)</td>
</tr>
<tr>
<td>Grade 3-4 hepatotoxicity</td>
<td>32 (7)</td>
<td>16 (5)</td>
<td>12 (6)</td>
</tr>
<tr>
<td>Fatigue (all grades)</td>
<td>195 (44)</td>
<td>153 (46)</td>
<td>89 (48)</td>
</tr>
<tr>
<td>Grade 3 fatigue</td>
<td>11 (2)</td>
<td>11 (3)</td>
<td>7 (4)</td>
</tr>
</tbody>
</table>
Conclusions: This exploratory analysis suggests the use of a CDK4/6 inhibitor in combination with an aromatase inhibitor for the first line treatment of HR+ MBC in older women results in similar efficacy benefit as seen in younger women. Although incidence and severity of Grade 1-4 adverse reactions appeared similar between age groups, greater serious adverse events and discontinuations occurred in patients ≥65. The inclusion of greater numbers of patients ≥70, in clinical trials will further inform clinicians about the safety and efficacy of CDK4/6 inhibitors in older adults.
Title: Weight change in postmenopausal women and breast cancer risk in the women's health initiative observational study

Rowan T Chlebowski¹, Juhua Luo², Garnet L Anderson³, Michael Simon⁴, Wendy Barrington⁵, Kerryn Reding⁵, JoAnn E Manson⁶, Thomas Rohan⁷, Jean Wactawski-Wende⁸, Dorothy Lane⁹, Howard Strickler⁷, Yasmin Mossavar-Rahmani⁷, Jo Freudenheim⁸, Abu Taiyab Nazmus Saquib¹⁰ and Marcia Stefanick¹¹.

¹City of Hope National Medical Center, Duarte, CA; ²Indiana University, Bloomington, IN; ³Fred Hutchinson Cancer Research Center, Seattle, WA; ⁴Karmanos Cancer Institute, Detroit, MI; ⁵University of Washington, Seattle, WA; ⁶Brigham and Women's Hospital, Harvard Medical School, Boston, MA; ⁷Albert Einstein College of Medicine, New York, NY; ⁸University at Buffalo, SUNY, Buffalo, NY; ⁹Stony Brook University School of Medicine, Stony Brook, NY; ¹⁰Sulaiman Al Rajhi College, School of Medicine, Al Bukayriyah, Saudi Arabia and ¹¹Stanford University School of Medicine, Stanford, CA.

Body: Purpose
While obesity is an established breast cancer risk factor, information about the influence of weight loss on breast cancer risk in postmenopausal women is mixed precluding generation of a strong public health message regarding potential benefits of weight loss with respect to cancer risk. Therefore, we evaluated associations between weight change and invasive breast cancer risk in postmenopausal women participating in the Women's Health Initiative (WHI) Observational Study.

Patients and Methods
Postmenopausal women (n=61,335) with no prior breast cancer and normal mammogram who were not underweight (body mass index [BMI] ≥ 18.5 kg/m²), ages 50-79 years at WHI enrollment between 1993 and 1998 at 40 US clinical centers, had body weight and height measured and BMI calculated at the clinical centers at baseline and at year 3. Weight change over 3 years was categorized as: stable (no change ≤ 5%), loss (change ≥ 5%), or gain (change ≥ 5%) with weight loss intentionality determined by self-report response to direct query at year 3. Breast cancers were initially ascertained through annual survey and were centrally confirmed by medical record review. Multi-variable Cox proportional hazards regression models incorporating breast cancer risk factors and baseline BMI were used to evaluate relationships between weight change and breast cancer incidence.

Results
During 11.4 years (mean) of follow-up, 3,061 women developed invasive breast cancer. In multi-variable analyses, compared with women with stable weight (n=41,139), women with weight loss (≥ 5%) (n=8,175) had a significantly lower breast cancer risk (hazard ratio [HR] 0.88 95% confidence interval [CI] 0.78-0.98). Adjustment for mammography did not alter findings (HR 0.88 95% CI 0.78-0.99). There was no significant interaction for breast cancer effect by weight loss intentionality determined by self-report response to direct query at year 3. Breast cancers were initially ascertained through annual survey and were centrally confirmed by medical record review. Multi-variable Cox proportional hazards regression models incorporating breast cancer risk factors and baseline BMI were used to evaluate relationships between weight change and breast cancer incidence.

Conclusion
Weight loss in postmenopausal women is associated with lower breast cancer risk. These findings suggest that postmenopausal women who lose weight may reduce their breast cancer risk.
**Title:** A validation of DCIS biological risk profile in a randomised study for radiation therapy with 20 year follow-up (SweDCIS)

Fredrik Wärnberg¹, Hans Garmo⁹, Yasin Folkvaljon¹, Lars Holmberg², Per Karlsson³, Kerstin Sandelin⁴, Steven Linke⁵, Stephen Lyle⁶, Karl Simin⁷, Glen Leesman⁵, Todd Barry⁷, Jess Savala⁹, Pat Whitworth⁹ and Troy Bremer⁵. ¹Uppsala University, Uppsala, Sweden; ²King’s College London, Medical School, Division of Cancer Studies, King’s College London, London, United Kingdom; ³Sahlgrenska University Hospital, Göteborg, Sweden; ⁴Karolinska Institutet, Stockholm, Sweden; ⁵PreludeDx, Laguna Hills, CA; ⁶University of Massachusetts Medical School, Worcester, MA; ⁷Spectrum Pathology, Mission Viejo, CA; ⁸Nashville Breast Center, Nashville, TN and ⁹Regional Cancer Centre, Uppsala University, Uppsala, Sweden.

**Body:**

**Background:** Women diagnosed with ductal carcinoma in situ (DCIS) and their physicians need tools that assess individualized risk and predict treatment benefit. A DCIS biologic signature was previously validated in an observational study at Kaiser Permanente NW. We evaluated the results of the signature for predictive utility in a national randomized clinical trial (SweDCIS) by assessing the 10-year benefit of adjuvant radiotherapy (RT) on ipsilateral breast event (IBE) and invasive breast cancer (IBC) risks.

**Methods:** The signature was validated in a prospective-retrospective study in women from the SweDCIS trial (n=1046) performed by the Swedish Breast Cancer Group. Women were treated with breast conserving surgery (BCS) between 1987-1999 and randomized to RT or no RT. A central pathology review of paraffin embedded tissue blocks (n=873) was performed at Uppsala University (UU). Freshly cut slides were provided to PreludeDx for biomarker testing. Extended follow-up of SweDCIS was published in 2014.

A panel of biomarkers (HER2, PR, Ki67, COX2, p16/INK4A, FOXA1 and SIAH2) were assayed and scored in PreludeDx’s CLIA lab by board-certified pathologists. Continuous Decision Scores (DS) were calculated with the biologic signature using the biomarker and clinical factors (age, size, margin, and palpability) blinded to patient outcome. The DS results were provided to the Uppsala Regional Cancer Center for analysis. A predefined and co-developed statistical analysis plan was executed. Absolute 10-year RT benefit was assessed using Kaplan-Meier survival analysis. Hazard ratios (HR) were determined using Cox proportional hazards analysis and the interaction of the DS and RT benefit was assessed.

**Results:** Complete biomarker and clinical information was available in 584 women. In women with clear margins (n=506), 78 IBEs, including 31 IBCs, were recorded within 10 years of diagnosis. The multivariate analysis of DS (0-10 unit scale) and the RT interaction was significant for risk of IBC (p=0.048) and IBE (p<0.001) at 10 years. The DS defined an elevated risk group (>3) for which there was pronounced 10-year benefit of RT (p=0.01) with an absolute risk reduction of 9% for IBC (Table 1). The corresponding low risk group (≤3), which included 48% of all patients, demonstrated no significant RT benefit (p=0.70) with an absolute risk reduction of 1%. The continuous DS variable was correlated with IBE risk, HR 1.49/per 5 units 95%CI [1.02,2.18] (p=0.038), in addition to the RT benefit for IBE in low (p=0.04) and elevated (p<0.001) risk groups.

**Discussion:** Evaluation of the SweDCIS trial validated prognostic and RT predictive utility of the biologic signature. Women diagnosed with DCIS and treated with BCS ±RT were stratified into clinically relevant low and elevated risk groups (≤3 vs >3). Women in the elevated risk group had twice the treatment benefit for IBC from RT compared to prior randomized trials, while the low risk group had no benefit from RT.

<table>
<thead>
<tr>
<th>DS Risk Groups</th>
<th>IBC events</th>
<th>In Situ or IBC events</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Absolute RT-benefit</td>
</tr>
<tr>
<td>Low Risk Group (DS≤3)</td>
<td>243</td>
<td>1%</td>
</tr>
<tr>
<td>Elevated Risk Group (DS&gt;3)</td>
<td>263</td>
<td>9%</td>
</tr>
</tbody>
</table>

Table 1. 10-year RT benefit in women from the SweDCIS trial.
Body: Background: The prediction of late distant recurrence (DR) is an important clinical goal for managing women with hormone receptor positive disease who have reached the end of 5 years' endocrine treatment without recurrence. Molecular profiles have produced conflicting results for the prediction of late DR. Here, we develop and validate a simple clinicopathological tool (Clinical Treatment Score post-5 years (CTS5)) to estimate the residual risk of DR after 5 years' endocrine treatment, which should help in discussions with patients about the potential benefits or not of continued endocrine therapy.

Patients and Methods: The ATAC dataset (N=4735) of postmenopausal women with oestrogen receptor (ER) positive breast cancer treated with 5 years' tamoxifen or anastrozole was used as a training cohort to establish a prognostic score for post-5-year risk of DR. CTS5 was based on five categories for nodal status, linear and quadratic terms for tumour size (capped at 30mm), three categories for grade, and continuous age. The validity of the CTS5 was tested in the BIG1-98 dataset (N=6711), which included postmenopausal women with ER-positive breast cancer treated with tamoxifen or letrozole (either monotherapy or sequential). Both cohorts included women who were alive and DR-free 5 years after randomization. Time to late DR, defined beginning at 5 years after ATAC or BIG 1-98 randomization, was the primary endpoint. Cox regression models estimated the prognostic performance of the CTS5. Hazard Ratios (HRs) are for a change of one Standard Deviation.

Results: The CTS5 model was a significant predictor for late DR in ATAC (HR=2.47 (95% CI, 2.24-2.73), P<0.001) and performed better than the established 0-10 year CTS model (Cuzick et al., JCO, 2011). CTS5 was confirmed as highly predictive for late DR in the BIG1-98 validation cohort (HR=2.07 (1.88-2.28), P<0.001). Of greatest importance was that CTS5 risk stratification defined in the training cohort as low (<5% risk of DR during years 5-10), intermediate (5-10% risk), high (>10% risk), identified 43% of the validation cohort as low risk, with an observed DR rate of 3.6% (95% CI 2.7-4.9) during years 5-10. Within nodal subgroups, 63% of node-negative were low risk with 3.9% (2.9-5.3) DR rate between years 5-10, and 24% having 1-3 nodes positive were low risk with 1.5% (0.5-3.8) DR rate between years 5-10. Separation of intermediate-risk from high-risk categories was also shown in the training set but improvements in calibration seem necessary for clinical utility for that assessment.

Conclusion: The CTS5 is a simple tool based on information that is readily available to all clinicians. It was more accurate in its prediction of DR risk in years 5-10 than the published CTS model. CTS5 was validated as highly prognostic for late DR in the independent BIG 1-98 study. The algorithm identified a subgroup of women with either node-negative disease or 1-3 positive nodes as having less than 1% per year risk of DR who could be advised of the limited value of extended endocrine therapy.
Title: The benefit of abemaciclib in prognostic subgroups: An exploratory analysis of combined data from the MONARCH 2 and 3 studies

Matthew P Goetz¹, Joyce O'Shaughnessy², George W Sledge Jr.³, Miguel Martin⁴, Yong Lin⁵, Tammy Forrester⁶, Colleen Mockbee⁶, Ian C Smith⁶, Angelo Di Leo⁶ and Stephen Johnston⁷. ¹Mayo Clinic, Rochester, MN; ²Baylor University Medical Center, Texas Oncology, Dallas, TX; ³Stanford University, Stanford, CA; ⁴Instituto De Investigacion Sanitaria Gregorio Marañon, Ciberonc, Geicam; Universidad Complutense, Madrid, Spain; ⁵Eli Lilly and Company, Indianapolis, IN; ⁶Hospital of Prato, Istituto Toscano Tumori, Prato, Italy and ⁷The Royal Marsden NHS Foundation Trust, London, United Kingdom.

Body: Background: Abemaciclib is an orally administered, selective inhibitor of cyclin-dependent kinases 4 & 6 that is dosed on a twice daily continuous schedule. Abemaciclib has demonstrated clinical efficacy with a generally tolerable safety profile in patients with hormone receptor-positive (HR+), human epidermal growth factor receptor 2-negative (HER2-) advanced breast cancer in combination with fulvestrant in MONARCH 2 (NCT02107703) and in combination with non-steroidal aromatase inhibitors (NSAI) in MONARCH 3 (NCT02246621). These analyses were conducted to evaluate if patient and disease characteristics may better inform in whom and when abemaciclib should be initiated to define optimal treatment strategies.

Methods: MONARCH 2 and 3 enrolled patients with HR+, HER2- advanced breast cancer. In MONARCH 2, patients whose disease had progressed while receiving endocrine therapy were treated with abemaciclib/placebo plus fulvestrant. In MONARCH 3, patients were treated with abemaciclib/placebo plus NSAI as initial therapy for advanced disease. An exploratory pooled analysis of the two studies was performed to determine significant prognostic factors. Efficacy results (progression-free survival [PFS] and objective response rate [ORR] in patients with measurable disease) were examined for patient subgroups corresponding to each of the identified significant prognostic factors. Subpopulation treatment effect pattern plot (STEPP) methodology was performed to examine the association between treatment-free interval (TFI) following adjuvant endocrine therapy and outcomes of endocrine therapy alone or in combination with abemaciclib in MONARCH 3.

Results: Analyses of clinical factors in over 1000 patients confirmed the following to have prognostic value: bone-only disease, liver metastases, tumor grade, progesterone receptor (PgR) status, and ECOG performance status. Prognosis was poor in patients with liver metastases, PgR-negative tumors, and high-grade tumors. While all subpopulations benefited from the addition of abemaciclib to endocrine therapy regardless of prognosis, substantial benefit of abemaciclib was observed in poor prognosis subgroups, characterized by large increases in PFS (hazard ratios = 0.4 to 0.5) and ORR (over 30%). In addition, STEPP analysis of TFI on a subset of the MONARCH 3 population showed that patients with the shortest TFI appeared to have a poorer prognosis and received more benefit from the addition of abemaciclib compared to patients with longer TFI.

Conclusions: This exploratory analysis has provided data that could help optimize treatment strategies by identifying that patients with poor prognostic factors may receive greater benefit from the addition of abemaciclib to endocrine therapy.
2017 San Antonio Breast Cancer Symposium

Publication Number: GS6-03

Title: Circulating tumor cells (CTCs) five years after diagnosis are prognostic for late recurrence in operable stage II-III breast cancer

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Body: **Background:** Late recurrence 5 or more years after diagnosis accounts for least one-half of all breast cancer recurrences, especially in hormone receptor (HR)+ disease. Biomarkers prognostic for late recurrence offer potential to more accurately identify subjects who might benefit from extended adjuvant endocrine therapy, or novel strategies to reduce late recurrence risk. **Methods:** CTCs were assessed at a single time point using the CELL SEARCH® assay in patients without clinical evidence of recurrence between 4.5-7.5 years after an initial diagnosis of HER2- stage II-III breast cancer and enrolled in trial E5103; all patients received surgery plus adjuvant chemotherapy and endocrine therapy for at least 5 years if HR+ disease. Patients were followed for evidence of clinical recurrence in accordance with standard care, and the association between CTCs and clinical recurrence was evaluated. **Results:** 546 patients without clinical evidence of recurrence enrolled between 2/25/13-7/29/16 and provided a blood sample that yielded a CTC result; 16 (2.9%) subsequently had a recurrence, of whom 15 had HR-positive disease. The median time between enrollment on E5103 and CTC assay was 5.2 years, and median/mean followup after the CTC assay was 1.6 years (range 0-3.9 years). The CTC assay was positive in 27 (4.9%) (median CTC count 1/7.5 ml blood, range 1-15). There were no significant differences in patient characteristics in the CTC+ vs. CTC- cases, including age < 50 years at initial diagnosis (52% vs. 44%), tumor size > 2 cm (63% vs. 59%), >/= 1+ node (81% vs. 72%), ER and/or PR+ (70% vs. 64%) or poor histologic grade (44% vs 55%). The recurrence rate per person-year in the CTC+ vs. CTC- groups was 19.6% vs. 1.1%, respectively (P<0.01). The median/time to recurrence after a positive CTC assay was 2.8 years. In multivariate analysis adjusted for clinical covariates (see table), a positive CTC assay was associated with an 18.3-fold increased risk of recurrence.

Univariate and Multivariate Analysis - Association Between CTC Status and Recurrence

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Hazard Ratio for Recurrence - Univariate Analysis (95% CI)</th>
<th>Hazard Ratio for Recurrence - Multivariate Analysis (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &lt; 50 years</td>
<td>3.4 (0.95-11.8)</td>
<td>3.0 (0.8-10.9)</td>
</tr>
<tr>
<td>Tumor size &gt; 2 cm</td>
<td>4.2 (0.98-19.2)</td>
<td>2.9 (0.6-13.2)</td>
</tr>
<tr>
<td>&gt;/= 1+ node</td>
<td>2.6 (0.59-11.5)</td>
<td>0.7 (0.1-3.8)</td>
</tr>
<tr>
<td>ER and/or PR+</td>
<td>8.3 (1.1-63.5)</td>
<td>7.7 (0.7-79.9)</td>
</tr>
<tr>
<td>Poor grade</td>
<td>0.43 (0.14-1.2)</td>
<td>1.2 (0.4-3.8)</td>
</tr>
<tr>
<td>CTC-pos vs CTC-neg</td>
<td>20.9 (7.5-58.3)</td>
<td>18.3 (5.7-58.2)</td>
</tr>
</tbody>
</table>

**Conclusions:** A single positive CTC assay in patients without clinical evidence of recurrence 5 years after diagnosis of stage II-III HR+, HER2- breast cancer provides independent prognostic information for late recurrence, providing proof of concept for using liquid-based biomarkers for late relapse risk assessment. These findings provide a foundation for further evaluation of this new risk assessment paradigm using CTC and other blood-based assays in this setting, and designing clinical trials to tailor therapeutic risk interventions. Supported by Breast Cancer Research Foundation (J. Sparano; R. Comis) and Susan G. Komen Foundation (J. Sparano), and by...
the National Cancer Institute (CA180820, CA180794, CA180790, CA180791, CA180795, CA180802, CA180816, CA180821, CA189859).
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Title: The EndoPredict score predicts residual cancer burden after neoadjuvant chemotherapy and after neoendocrine therapy in HR+/HER2- breast cancer patients from ABCSG 34

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Body: Background:
In the neoadjuvant treatment setting of ER+/HER2- breast cancer (BC) the choice between preoperative endocrine - (NET) or preoperative chemotherapy (NCT) is largely based on the expression of hormone receptors (HR), grading and possibly Ki-67. The EndoPredict 12-gene molecular score (EP) is a validated prognostic score based on the expression of genes involved in cellular processes including proliferation and ER signaling/differentiation. The application of the EP molecular score may thus provide additional insight into neoadjuvant response of tumors.

Methods:
This prospective translational study was carried out as an exploratory endpoint within the ABCSG 34 protocol (SABCS 2016, Singer et al.) and included only the HR+ subset of women. ABCSG 34 was a randomized 2-arm academic phase-II trial including 400 patients (250 ER+) with HER2 negative early BC. Patients were selected for either NET or NCT according to the study protocol reflecting standard of care: postmenopausal women with ER+++, or ER++ and Ki67 <14%, and G1,2,X tumors received 6 months of Letrozole. Postmenopausal patients with ER-(and PgR+) or ER low and Ki67 ≥14%, and with G3 tumors, and premenopausal patients, received 8 cycles of anthracycline/taxane based chemotherapy. Primary endpoint was Residual Cancer Burden score (RCB0/I vs RCBII/III) at surgery in women with or without Tecemotide (L-BLP25). EP was assessed from diagnostic cores and the predefined risk groups (EP low-risk vs EP high-risk; cutoff value: 5.0) and the score as a continuous variable (EPc) were applied to all patients with both full molecular - and RCB data (n=217).

Results:
134 patients (EP low-risk:9; EP high-risk:125) with HR+ tumors received NCT. Stage I: 14.9%, II/III: 84%; G3: 56%; mean Ki-67: 40.4%. No patient with EP low-risk score showed RCB0/I (NPV: 100%) whereas 26.4% of EP high-risk showed full response. In logistic regression EP as a continuous score (EPc) was a significant predictor of RCB0/I, showed an AUC (95% CI) of 0.736 (0.63 - 0.84) and revealed close correlation with Ki-67.

83 patients (EP low-risk:44; EP high-risk:39) received NET. Stage I: 38.6%, II/III: 60.2%; G3: 4.8%; mean Ki-67: 14.9%. 27.3% (n=12/44) with EP low-risk and 7.7% (n=3/39) with EP high-risk showed RCB0/I. EPc was a significant predictor of RCB0/I with an AUC (95% CI) of 0.726 (0.60 - 0.85). In all multivariate analyses low anatomic stage was the most powerful predictor of RCB0/I.

Conclusions:
Clinical standard of care separated ABCSG 34 patients into two HR+ cohorts with differing clinical features and treated distinctly with either NET or NCT: In women treated with neoadjuvant Letrozole a high molecular score indicated a very low possibility of endocrine response. In women clinically selected for neoadjuvant chemotherapy, a high EP score was associated with a higher
probability of chemotherapy response and the low EP risk group outlined a group of women with very poor tumor response to NCT (NPV 100%).

In summary, EP low risk is associated with tumor response to endocrine treatment and predicts resistance in the chemotherapy group. NCT, especially to attain breast conservation in ER+/HER2-/EP low risk should be reconsidered.
Title: Gain-of-function kinase library screen identifies FGFR1 amplification as a mechanism of resistance to antiestrogens and CDK4/6 inhibitors in ER+ breast cancer

Luigi Formisano¹, Yao Lu¹, Valerie M Jansen¹, Joshua A Bauer¹, Ariella Hanker¹, Paula Gonzalez Ericsson¹, Kyung-Min Lee¹, Melissa J Nixon¹, Angel L Guerrero-Zotano¹, Luis J Schwarz¹, Melinda Sanders¹, Dhivya Sudhan¹, Teresa C Dugger¹, Marcelo Rocha Cruz², Amir Behdad², Massimo Cristofanilli², Aditya Bardia³, Joyce O'Shaughnessy⁴, Ingrid A Mayer¹ and Carlos L Arteaga¹. ¹Vanderbilt Medical Center; ²Robert H Lurie Comprehensive Cancer Center; ³Massachusetts General Hospital Cancer Center and ⁴Baylor University Medical Center.

Body: Background: CDK4/6 inhibitors have been approved in combination with endocrine therapy for treatment of ER+ metastatic breast cancer. The goal of this study was to discover mechanisms of resistance to ER antagonists alone and in combination with CDK4/6 inhibitors.

Results: To achieve this goal, we used lentiviral vectors to individually express 559 human kinase open reading frames (ORFs) in ER+ MCF7 human breast cancer cells treated with fulvestrant ± the CDK4/6 inhibitor ribociclib. In MCF7 cells treated with fulvestrant alone or with ribociclib, we identified 15 and 17 kinases associated with resistance, respectively. Ten of these kinases overlapped in both groups. In a secondary screen, MCF7 cells were stably transduced with V5-tagged lentiviruses expressing the positive 'hits' for treatment with fulvestrant/ribociclib. Five of 17 kinases (FGFR1, FRK, HCK, FGR, CRKL) were confirmed to induce resistance to fulvestrant/palbociclib and fulvestrant/ribociclib. Survey of TCGA for copy number alterations and/or expression of these 5 genes showed only FGFR1 to be amplified/overexpressed in ~15% of ER+ breast cancers. Experiments in vitro showed that ER+/FGFR1-amplified (amp) MDA-134, CAMA-1 and HCC1500 human breast cancer cells and MCF7 cells stably transduced with FGFR1 were relatively resistant to estrogen deprivation, fulvestrant and fulvestrant/palbociclib compared to non-FGFR1 amp MCF7 cells. This resistance was abrogated by treatment with the FGFR tyrosine kinase inhibitor (TKI) lucitanib. Treatment with fulvestrant or palbociclib alone modestly delayed growth of ER+/FGFR1-amp breast cancer patient-derived xenografts (PDX) established in nude mice. However, addition of the FGFR TKI erdafitinib to fulvestrant/palbociclib resulted in marked PDX regression in all mice without associated toxicity and a complete cell cycle arrest measured by Ki67. Treatment of FGFR-amp cells with FGF-2 strongly induced CCND1 (cyclin D1) expression. Downregulation of CCND1 with CCND1 RNAi oligonucleotides restored sensitivity of FGFR1-amp cells to fulvestrant/palbociclib, thus phenocopying the effect of FGFR TKIs. Conversely, overexpression of CCND1 in MCF7 cells induced resistance to estrogen deprivation and to fulvestrant ± palbociclib. Finally, we examined next gen sequencing of cell free tumor DNA by Guardant360 in 34 patients before and after progression on CDK4/6 inhibitor. In 10/34 (29%) post-progression specimens, we detected alterations in the FGFR pathway: FGFR1 amplification (n=7), FGFR1 N546K (n=1), FGFR2 N549K (n=1), and FGFR2 V395D (n=1) activating mutations.

Conclusions: These data suggest aberrant FGFR signaling is a mechanism of resistance to anti-ER therapies ± CDK4/6 inhibitors. We posit overexpression of cyclin D1 induced by both FGFR signaling and ER transcription plays a role in drug resistance. Based on these findings we propose ER+/FGFR1 amplified breast cancers are endocrine resistant and should be candidates for treatment with combinations of ER and FGFR antagonists. Accordingly, we have initiated a phase Ib trial of fulvestrant, palbociclib and erdafitinib in patients with antiestrogen resistant ER+/HER2-negative breast cancer with FGFR1-4 amplification.
Title: Identifying metastatic drivers in patient-derived xenograft models of triple negative breast cancer

Emily Powell¹, Jiansu Shao¹, Hector M Picon¹, Zhongqi Ge¹, Gloria V Echeverria¹, Michael Peoples¹, Christopher Bristow¹, Shirong Cai¹, Yizheng Tu¹, Aaron M McCoy¹, David Piwnica-Worms¹, Giulio Draetta¹, John R Edwards², Stacy L Moulder¹, William F Symmans¹, Timothy P Heffernan¹, Han Liang¹ and Helen Piwnica-Worms¹. ¹MD Anderson Cancer Center and ²Washington University in St. Louis.

Body: Metastases are responsible for the vast majority of deaths due to breast cancer. Triple negative breast cancer (TNBC) is an aggressive subtype of breast cancer characterized by high rates of metastasis and poor prognosis. We are employing patient derived xenograft (PDX) models of TNBC to identify drivers of metastasis. Tumor samples are obtained from the breast tumors of patients with TNBC and engrafted immediately into the humanized mammary fat pads of immune compromised mice. Lentiviral transduction was employed to express bioluminescent and fluorescent markers in two independent PDX models of TNBC. Using these models, we demonstrated that human breast tumors are capable of completing all stages of the metastatic cascade in mice, and metastatic lesions are observed in organs normally found in patients with metastatic breast cancer including lung, liver, bone, brain, and lymph nodes. Dynamic and reversible epithelial to mesenchymal transition (EMT) was observed as tumors metastasized to lung and were re-passaged to recipient mouse mammary glands. Lung metastases were isolated using bioluminescence imaging and lung metastasis gene expression signatures were generated. Metastasis signatures from two independent PDX models were compared to identify genes that were commonly de-regulated in lung metastases relative to corresponding mammary tumors. Comprehensive gain-of-function screens were then conducted in vivo to identify functional drivers of TNBC metastasis. Carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5) was identified as a metastatic driver in this screen. CEACAM5 mRNA and protein levels were elevated in lung metastases relative to corresponding mammary gland tumors in mice. In addition, we demonstrated that CEACAM5 expression was upregulated in the lung metastases of breast cancer patients, and its expression inversely correlated with patient survival. Our data indicate that the metastatic function of CEACAM5 is to promote growth of breast tumors in the lung by inducing MET (mesenchymal to epithelial transition).
EMBRACA: A phase 3 trial comparing talazoparib, an oral PARP inhibitor, to physician’s choice of therapy in patients with advanced breast cancer and a germline BRCA mutation

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Body: Background: Talazoparib (TALA) is a highly potent, dual-mechanism PARP inhibitor that inhibits the PARP enzyme and effectively traps PARP on single-stranded DNA breaks, preventing DNA damage repair and causing cell death in BRCA1/2-mutated cells.

Methods: EMBRACA is an open-label, randomized, 2-arm, phase 3 trial comparing the efficacy and safety of TALA (1 mg/day) with standard single-agent physician’s choice of therapy (PCT) (capecitabine, eribulin, gemcitabine, or vinorelbine) in patients with advanced breast cancer (aBC) and a germline BRCA1/2 mutation (gBRCAmut). The primary objective was PFS assessed by blinded independent central review (BICR). Secondary objectives: OS, ORR, CBR at 24 weeks (CBR24), and safety. Exploratory objectives: patient-reported QoL and DOR. Eligibility criteria: age ≥ 18 years; HER2-negative aBC; deleterious or suspected deleterious gBRCAmut; ≤ 3 prior cytotoxic regimens for aBC; and ECOG PS ≤ 2. Prior platinum was allowed. Patients were randomized 2:1 and stratified by receptor status, extent of prior therapy, and CNS metastases (NCT01945775).

Results: 431 patients were randomized (median age 46 years; 54% hormone-receptor [HR]+ BC; 45% BRCA1+ and 55% BRCA2+; 55% ECOG PS = 0; 38% chemo-naïve for aBC; 18% prior platinum; 15% CNS metastases); 287 were assigned to TALA and 144 to PCT (1 TALA, 18 PCT patients were not treated). Median duration of exposure was 6.1 and 3.9 months, respectively; TALA had a relative dose intensity of 87%. At 62% PFS data maturity:

<table>
<thead>
<tr>
<th>TALA</th>
<th>PCT</th>
<th>Hazard Ratio/Odds Ratio (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFS by BICR, mo (95% CI)</td>
<td>8.6 (7.2-9.3); n = 287</td>
<td>5.6 (4.2-6.7); n = 144</td>
</tr>
<tr>
<td>OS [interim], mo (95% CI)</td>
<td>22.3 (18.1-26.2); n = 287</td>
<td>19.5 (16.3-22.4); n = 144</td>
</tr>
<tr>
<td>ORR by INV, % (95% CI)</td>
<td>62.6% (55.8-69.0); n = 219</td>
<td>27.2% (19.3-36.3); n = 114</td>
</tr>
<tr>
<td>DOR by INV, mo (IQR)</td>
<td>5.4 (2.8-11.2); n = 137</td>
<td>3.1 (2.4-6.7); n = 31</td>
</tr>
<tr>
<td>CBR24 by INV, % (95% CI)</td>
<td>68.6% (62.9-74.0); n = 287</td>
<td>36.1% (28.3-44.5); n = 144</td>
</tr>
</tbody>
</table>

INV, investigator. *Not a randomized subset.

Improved clinical benefit was seen in all subsets including those with HR+ BC (HR 0.47; 95% CI 0.32-0.71) and CNS metastasis (HR 0.32; 95% CI 0.15-0.88). There was a significant delay in the time to deterioration in global health status (GHS/QoL for TALA vs PCT (HR 0.38; 95% CI 0.26-0.55; P < 0.0001). Grade 3-4 hematologic adverse events (AEs) occurred in 55% TALA (mainly anemia)/39% PCT (mainly neutropenia). Grade 3-4 non-hematologic AEs were seen in 32% TALA/38% PCT; TALA was associated with fewer gastrointestinal disorders (5.6% vs 11.9%) and skin/subcutaneous tissue disorders (0.7% vs 5.6%) than PCT. Grade 3-4 serious AEs were observed in 26% TALA/25% PCT. AEs associated with permanent study drug discontinuation occurred in 8% TALA/10% PCT. AE resulting in death occurred in 2.1% TALA/3.2% PCT. Conclusions: Single-agent TALA significantly prolonged PFS by BICR in HER2-negative aBC patients with a gBRCAmut compared to PCT; all key secondary efficacy endpoints demonstrated benefit with TALA, with a significant delay in time to deterioration in GHS/QoL.

TALA was
generally well tolerated with minimal non-hematologic toxicity and few AEs associated with treatment discontinuations.
Body: Objectives: Brain metastases of breast cancer demonstrate low and heterogeneous levels of permeability to drugs in mouse models and human craniotomies. The Blood-Brain Barrier (BBB), the protective lining of CNS blood vessels, impedes drug entry into the normal brain. When a metastasis forms, the BBB is locally altered to a poorly characterized Blood-Tumor Barrier (BTB). Quantitative experimental models indicate that most brain metastases have increased permeability over the normal BBB, but BTB permeability is both heterogeneous and ~2 logs less than that of systemic metastases. We have interrogated three hematogenous models of brain metastasis of breast cancer to ask (1) whether the BTB is an ordered structure or a random breakdown of the BBB; (2) among brain metastases, whether consistent differences underlie the BTBs of lesions with low- and high permeabilities to fluorescent markers and drugs; (3) if alterations in BTB composition can functionally change its permeability. Our long term goal is to enhance uptake of drugs into brain metastases to effective levels.

Results: When uninvolved brain was compared with any brain metastasis, alterations in endothelial, pericytic, astrocytic, and microglial components of the BBB were observed. Both the pericyte and astrocyte components of the BTB were consistently altered with increased permeability: When metastases with relatively low and high permeability were compared, increased expression of a desmin+ subpopulation of pericytes was associated with higher permeability (231-BR6 P=0.0002; JIMT-1-BR3 P = 0.004; SUM190-BR3 P=0.008). A trend toward reduced CD13+ pericytes was observed in highly permeable metastases (231-BR6 P =0.014; JIMT-1-BR3 P =0.002, SUM190-BR3, NS). For GFAP+ astrocytes in the neuroinflammatory response surrounding metastases, no overall difference in cell number was observed between low and high permeability lesions. However, gene expression profiling of laser capture microdissected low and high permeability lesions demonstrated overexpression of the sphingosine-1 phosphate receptor 3 (S1P3) in the astrocytes of highly permeable lesions, which was confirmed at the protein expression level in all three models (231-BR6 P=0.034; JIMT-1-BR3 P = 0.01; SUM190-BR3 P=0.016). Inhibition of S1P3 via S1PR3 shRNA or a selective antagonist (TY-52156) functionally tightened the BTB in an in vitro model. Administration of TY-52156 to mice harboring 231-BR6 brain metastases had no effect on metastasis number, but decreased uptake of Texas Red Dextran dye into metastases (P=0.016). S1P3 mediated its effects on BTB permeability through astrocytic secretion of IL-6 and CCL2, which altered endothelial expression and localization of adhesive proteins, a potentially translatable pathway. Both desmin+ pericytes and S1P3+ astrocytes are present in human craniotomy specimens.

Conclusions: These experiments demonstrate that the BTB is a structure with consistent properties, and that further consistent changes underlie the transition from a low to high permeability BTB. While proof of principle, S1P3 inhibition studies indicate that the BTB permeability can be functionally modulated in vivo.
Title: Targeting PTK6 to treat mesenchymal triple negative breast cancer

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Body: Background/Rational: Patients with triple negative breast cancers (TNBC) have limited therapeutic options beyond conventional chemotherapy. Unfortunately, high-risk for metastatic recurrence and chemotherapy resistant diseases cause the worst 5-year survival rate in patients with TNBC, which have been significant clinical challenges. Novel therapeutic targets or strategies to combat metastasis and chemotherapy resistance are necessary to improve quality of life and outcomes for patients with high risk TNBC. Epithelial-to-mesenchymal transition (EMT) and anoikis resistance are processes recognized as contributing to enhanced metastatic potential and treatment resistance. A subset of TNBC exhibits mesenchymal gene signatures and phenotypes that may be associated with high metastatic recurrence, chemotherapy resistance and immunosuppression. In a functional genomic screen, we identified several candidates as novel regulators of EMT and anoikis sensitivity of TNBC cells. We have focused on roles of one highly validated candidate, protein tyrosine kinase 6 (PTK6) on EMT, anoikis resistance and metastatic capacity in TNBC. Methods: We analyzed expression of PTK6 and mesenchymal markers in patient triple negative tumors by immunohistochemistry. In breast epithelial and TNBC cell lines, the levels of PTK6 were genetically modulated, and determined effects on growth, migration and EMT. In vivo mouse models were used to show effects of PTK6 inhibition on metastatic capacity of TNBC cells. We have also validated effects of PTK6 specific small molecule inhibitor on TNBC growth and metastases. In order to dissect specific mechanisms by which PTK6 inhibition regulates TNBC mesenchymal phenotypes, we used a siRNA library screening and identified novel E3 ligases that may be responsible for PTK6 inhibition-induced EMT regulation. Results: Overexpression of PTK6 in MCF10A cells is sufficient to promote an EMT; promotes migration, suppresses epithelial markers (E-cadherin/claudin-1) and increases mesenchymal markers (N-cadherin and fibronectin). In contrast, PTK6 inhibition either PTK6 shRNAs or treatment with a specific kinase inhibitor enhances E-cadherin expression and suppresses migration, anoikis resistance and lung colonization of TNBC cells. PTK6-dependent E-cadherin regulation is specifically dependent on levels of SNAIL, a transcriptional repressor that is associated with poor TNBC patient prognosis. SNAIL down-regulation by PTK6 inhibition is directly responsible for the modulation of anoikis sensitivity, which is in turn causally linked to lung colonization potential. PTK6 inhibition promotes the proteasome-dependent degradation of SNAIL via a novel mechanism independent of GSK3β/β-TRCP pathway or Fbox E3ligases (FBXO5, FBXO11, FBXL14) that are known to regulate SNAIL ubiquitination. Using a siRNA library screening approach, we identified novel E3 ligase candidates that may be responsible for SNAIL ubiquitination and degradation downstream of PTK6 inhibition. Conclusion/Future direction: PTK6 is a representative novel regulator of EMT and anoikis resistance that can be targeted to prevent metastases of TNBC. Modulation of mesenchymal phenotypes of TNBC cells may be able to regulate chemotherapy resistance and/or immunosuppressive microenvironment.
Title: Preclinical evidence that distant metastases occur via the lymphatic route

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Body: Cancer that metastasizes beyond the lymph nodes is almost invariably terminal. Therefore, understanding how cancer metastasizes is a critical aspect to understanding and eventually preventing cancer spreading from tissue to tissue. A major question that remains controversial is whether cancer cells that metastasize to the LNs are able to physically leave the LNs to colonize distant tissues. Moreover, whether cancer cell metastasis between different tissues involves unique genes is a question that requires further research. Using the metastatic mouse breast carcinoma line 4T1 in an LN microinjection model, we provide evidence suggesting that cancer cells can indeed metastasize and colonize distant tissues after direct implantation into the LNs. Interestingly, direct comparison of distant tissue metastatic capability comparing LN-microinjected cells and tail vein-injected cells suggests that the lymphatic route of cancer cell spreading may be a more efficient mechanism of metastasis than the hematogenous route. Moreover, our comparative transcriptome analysis results suggest that LN metastases show unique gene expression profiles relative to the primary breast tumors and distant metastases. We identified several genes that are up- or down-regulated in an LN-specific manner and thus may play a role in metastasis to the LNs and beyond. Collectively, our results suggest that LN metastases constitute an active “staging site” from which breast cancer cells can further metastasize to distant sites. Furthermore, we identify a unique set of genes that may be involved in the breast cancer metastatic process, several of which are potentially targetable using currently available inhibitors.
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**Title:** Mutational profiling of breast cancer brain metastases – matched pair analysis of next generation sequencing between primary breast cancer and later developed brain metastases

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**Body: Background:** Despite increased survival in patients with advanced breast cancer, breast cancer brain metastases (BCBM), remains a final frontier with a mean survival of 3-12 months. The biology facilitating BCBM is not fully understood.

**Aims:** To compare gene expression profiles in primary BC, and later diagnosed, surgically removed BCBM.

**Material and Methods:** A total of 58 BCBM and 46 breast tumors were analyzed with next generation sequencing (NGS). DNA was isolated from FFPE sections and then the Cancer Hotspot Panel v2 (ThermoFisher Scientific) covering 207-targeted regions in 50 cancer related genes was used. Template preparation and enrichment were performed with the IonChef™ Instrument (ThermoFisher Scientific). Eight barcoded samples were pooled per Ion 318™ v2 BC chip and sequenced on the Ion PGM™ System (ThermoFisher Scientific). Alignment to the hg19 human reference genome and variant calling was performed by the Torrent Suite Software v5.2.0 (ThermoFisher Scientific) also IonReporter™ System (ThermoFisher Scientific) was used.

**Results:** 46 of the BCBM were matched with a primary breast cancer tumor. All but 12 tumors had the same IHC characteristic in the matched pairs. The most common transformation was Luminal A to Luminal B in 8 tumors. The other changes were triple negative subtype (TNBC) to Luminal B, HER2+ to Luminal B, Luminal A to TNBC and Luminal A to HER2+ with one case respectively. The BCBM had the following IHC profile: one tumor was luminal A (1%), 15 tumors were Luminal B (25%), 29 were TNBC (50%) and 14 HER2 overexpressing (24%). The preliminary NGS data shows that the most common mutation in BCBM was found in the tumor suppressor gene p53 (22/58, 38%). Other common mutations were PIK3CA (17/58, 29%); KDR (16/58, 28%), KIT (9/58, 16%) and PTEN (2/58, 3%). The corresponding figures in the primary BC were p53 (15/46, 33%), PIK3CA (16/46, 35%), KDR (17/46, 37%), KIT (7/46, 15%) and no tumors with PTEN. The mutational spectra in the 50 cancer related genes were similar in the primary BC as in the BCBM with 1-5 different driver mutations but additional mutations were registered in 6/46 matched cases (13%). We fail to identify any specific differences in mutations between the different morphological subtypes (LumA/LumB/TNBC/HER2+) in the metastatic sample. In the NGS analysis of the metastases and primary tumors 3 different variations of p53 was detected.

**Conclusions:** In this large matched pairs of primary breast tumors and BCBM we show that the majority of BCBM have a similar gene profile as the primary BC. The most common aberrations were found in TP53, PIK3CA and KDR. Additional post analyses are under investigation and will be added to the results. It appears that brain metastases are not different from other metastases in that they remain fairly stable in their driver mutational profile. When the mutational profile changes there is addition of mutation rather than deletion. A clinical implication of these results could be to treat BCBM according to the mutational profile of the primary tumor which decreases the need for sampling of BCBM; something that if the patient is not eligible for surgery might otherwise prove complex.
Title: Wnt5a induces ROR1 to associate with cortactin, which undergoes tyrosine phosphorylation, and enhances migration of breast cancer cells

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Body: Background: ROR1 is an evolutionarily conserved, oncoembryonic surface-antigen expressed in breast cancer. Previously, we found that ROR1 can serve as a receptor for Wnt5a, which can induce non-canonical Wnt signaling that enhances cancer-cell migration. Recently we found that Wnt5a could induce ROR1 to complex with a cytoskeletal protein designated HS1, which recruited ARHGEF1, enhanced activation of RhoA, and promoted leukemia-cell migration. These effects of Wnt5a on CLL cells could be inhibited by cirtuzumab, a humanized, high-affinity monoclonal antibody (mAb) specific for ROR1. As expression of HS1 is limited to hematopoietic cells we examined breast cancer cell lines and primary breast cancer cells for expression of cortactin. Cortactin (also called EMS1), is a cytoskeletal protein, which is homologous in structure and function to HS1 and is broadly expressed in human cancers, including breast cancer.

Methods: We performed immunoprecipitation studies using high-affinity mAb to ROR1 or cortactin and immunoblot analysis to examine for the association of ROR1 with cortactin and tyrosine phosphorylation of cortactin at Y421. We also assessed the expression levels of cortactin, ROR1, and ARHGEF1, in the MCF7 breast cancer cell line and in breast-cancer patient-derived xenografts (PDX). We also generated MCF7-ROR1 cells, which were stably transfected to express ROR1 or various mutant forms of ROR1 generated to study the structure-function requirements for effective ROR1-cortactin interactions.

Results: We found that ROR1 associates with cortactin in freshly-isolated breast cancer PDX tumors or in PDX cells cultured with exogenous Wnt5a. We corroborated these results in breast cancer cell line MCF7-ROR1 cells, which were transfected to express ROR1 and found to migrate more effectively than the parental MCF7 lacking expression of ROR1. Wnt5a also induced cortactin tyrosine phosphorylation at Y421, recruitment of ARHGEF1, and activation of RhoA, which we found associated with enhanced breast-cancer cell migration. The capacity of Wnt5a to induce such changes could be blocked by treatment of the cells with cirtuzumab. We generated truncated forms of ROR1 without a proline-rich domain (PRD) and found PRD was necessary for this association or Wnt5a-induced cortactin phosphorylation and enhanced cancer-cell migration. Accordingly, we introduced single amino-acid substitutions of proline (P) to alanine (A) in the ROR1-PRD at positions 784, 808, 826, or 841 in potential SH3-binding motifs. In contrast to wild-type ROR1, or other ROR1P→A mutants, ROR1P(841)A had impaired capacity to recruit cortactin and ARHGEF1 to ROR1 in response to Wnt5a. Moreover, Wnt5a could not induce cells expressing ROR1P(841)A to phosphorylate cortactin or activate ARHGEF1, and was unable to enhance the motility of the MCF7 cells transfected with this mutant form of ROR1.

Conclusions: Collectively, these studies indicate the capacity of cortactin to complex with ROR1 plays an important role in ROR1-dependent Wnt5a-enhanced breast cancer cell migration. These studies also demonstrate that cirtuzumab can inhibit the formation of cortactin-ROR1 complexes, cortactin phosphorylation, and cancer-cell migration, and metastasis.
Title: Targeting the SphK1/S1P/S1PR1 axis that connects obesity, chronic inflammation, and breast cancer metastasis

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Body: Introduction: Obesity with associated inflammation is now recognized as a risk factor for breast cancer and increased incidence of distant metastases. However, the link between obesity and breast cancer progression remains poorly understood. There is growing evidence that sphingosine-1-phosphate (S1P), a pleiotropic bioactive sphingolipid metabolite enriched both in blood and lymphatic fluid is involved in inflammation, obesity, and breast cancer progression. Our hypothesis is that obesity increases levels of S1P in both tumor and its microenvironment, which play a role in obesity-induced inflammation and breast cancer metastasis. The aim of this study is to test this hypothesis in in vitro and in vivo as well as patient settings.

Methods: Levels of sphingolipids including S1P in serum from breast cancer patients were quantified. Orthotopically-implanted E0771 syngeneic breast cancer and MMTV-PyMT transgenic breast cancer mouse models were used. Mice were fed with normal or high-fat diet (HFD). FTY720 was administered orally (1 mg/kg/day). To examine pre-metastatic niche formation, a mouse model utilizing tail vein injection of E0771 cells was used. In this model, mice were treated with conditioned media from E0771 breast cancer cells overexpressing SphK1 (K1-CM) or that from E0771 cells cultured with the vector control (CT-CM), prior to tail vein injections of naive E0771 cells. S1P levels were determined by electrospray ionization-tandem mass spectrometry.

Results: We found that obesity significantly increased S1P levels in serum from breast cancer patients. In animal breast cancer models, HFD upregulated expression of sphingosine kinase 1 (SphK1), the enzyme that produces S1P, and its receptor S1PR1 in syngeneic and spontaneous breast tumors. HFD also significantly increased S1P in breast tumors and in the tumor interstitial fluid, which is a component of the tumor microenvironment and bathes cancer cells in the tumor. Targeting the SphK1/S1P/S1PR1 axis with FTY720/fingolimod attenuated obesity-induced key pro-inflammatory cytokines, macrophage infiltration, and tumor progression. In addition, S1P produced by tumor SphK1 primed lung pre-metastatic niches, increased macrophage recruitment into the lung, and induced IL-6 and signaling pathways important for lung metastatic colonization. FTY720 suppressed HFD-induced lung IL-6 and macrophage infiltration as well as S1P-mediated signaling pathways and dramatically reduced formation of metastatic foci. In tumor bearing mice, FTY720 also suppressed obesity-related inflammation, S1P signaling, pulmonary metastasis, and prolonged survival.

Conclusion: Our results highlight a critical role for circulating S1P produced by tumor and the SphK1/S1P/S1PR1 axis in obesity-related inflammation, metastatic niche formation and breast cancer metastasis and suggest that targeting the SphK1/S1P/S1PR1 axis would be a useful therapeutic for obesity promoted metastatic breast cancer.
Clinical and histological characteristics of peritoneal metastases of invasive lobular breast cancer

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Body: Introduction: In previously reported autopsy series, peritoneal metastases have been detected in up to 40% of patients with invasive lobular breast cancer (ILC). Despite modern diagnostic techniques such as high-resolution ultrasound, it remains a challenge to differentiate ovarian cancer from metastatic breast cancer with peritoneal manifestation before or during surgery. The goal of this analysis was to determine typical clinical and immunohistological features of peritoneal metastasis of ILC. Specifically, we asked the question whether there are predictive factors in primary breast cancer associated with subsequent development of peritoneal metastasis. Patients and methods: We identified 58 patients with ovarian metastases in the Charité cancer register (4,792 breast cancer patients from 2003 to 2015). We looked for clinical and pathological differences between breast cancer patients with (N=58) and without (N=4734) peritoneal metastases and between ILC and non-ILC breast cancer subtypes. Imaging and surgical reports of these 58 patients with ILC intraperitoneal metastases were reviewed. Results: The majority (84.7%) of primary breast cancers consisted of subtypes other than ILC and only 15.3% were histologically characterized as ILC. In contrast, 63.6% of patients with peritoneal metastases had histologically proven ILC in the metastatic tissue. Other subtypes where found in the 36.4% of the metastatic tissue (p<0.001). The Odds ratio for peritoneal metastases for ILC was 2.35 (95% CI 1.655-3.332) and for Non-ILC 0.23 (0.185-0.284). There were no significant differences in receptor status between primary and peritoneal metastatic ILC. Comparing ER/PR expressions levels on primary tumor versus metastasis, while statistically not significant (p=0.805), showed a rise in ER expression in 42.95% in the metastatic tissue while PR expression remained stable with no difference in 53.3% and a rise in the metastatic site in only 26.7% (p=0.715). Median age of all patients with primary breast cancer was 60 years (10%-90%: 41-75). There was a significant difference in age at diagnosis of metastasis between patients with (50.5 years) and without peritoneal metastases (59 years) (p= 0.002). Median time to development of peritoneal metastases for all patients was 48.5 months (10%-90%: 0-191.7), for ILC 44 months (0-198.2) and for Non-ILC 56.5 months (6.7-206.4) (p= 0.487). Median survival time for patients with ILC and peritoneal metastases was 56 months, for Non-ILC 53 months (p=0.759). 33 of 58 patients had radiologic evidence of disease, 26 with ILC and 7 with Non-ILC. An ovarian mass was detected by imaging in 15/26 patients with ILC and 4/7 patients with non-ILC. Ascites and diffuse peritoneal metastases were seen in 18/26 patients with detectable ILC and in 4/7 patients with Non-ILC. Conclusion: This is the first comprehensive analysis of clinical and pathological characteristics of peritoneal metastases showing ILC is more frequent than other histologic subtypes. Patients with peritoneal metastasis are significantly younger (median 50.5y.) than patients without. The clinical signs are similar to those of ovarian cancer except and therefore the diagnosis of metastatic ILC must be taken into consideration as a differential diagnosis.
Title: DPYSL3 modulates proliferation and migration in claudin-low breast cancer

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Body: Cancer is often driven by deregulated protein kinases pathways including breast cancer. To profile the kinome at the protein level we are developing a kinase pulldown (KiP) proteomic platform that uses multiple kinase inhibitors conjugated to sepharose beads for protein kinase profiling. Mass spectrometry-base tryptic peptide sequencing is used to identify and quantify the kinome in an unbiased manner. To date, we have profiled and analyzed the kinomes of more than 50 breast cancer PDX model systems that include all five breast cancer subtypes. In these analyses we have begun to explore the kinome interactome because we have observed that KiP also pulls down breast cancer subtype specific kinase interacting proteins that may represent new biological insights. For example we have consistently identified dihydropyrimidinase-like 3 (DPYSL3) in p21-activated kinase (PAK) inhibitor KiP interactome in the claudin-low WHIM12 PDX model.

DPYSL3 is an intracellular phosphoprotein known to play a role in cell migration and metastasis. However it remains to be seen if DPYSL3 functions as a suppressor or a promoter of metastasis. We therefore generated DPYSL3 knock-down cell lines to evaluate function in claudin-low breast cancer. Proliferation levels of DPYSL3 depleted Claudin-low WHIM12 cells (DPYSL3-) were lower than those of control WHIM 12 (DPYSL3+) cells. In contrast, migration levels of DPYSL3- WHIM12 were greater than those of control cells, suggesting a pivotal role for DPYSL3 in several cellular physiologies. Additionally the activity of a PAK inhibitor (FRAX597) on migration appeared to require DPYSL3, as the inhibitory effect of FRAX579 on migration in WHIM12 was lost when DPYSL3 expression was depleted.

Additionally we find that only the long isoform of DPYSL3, not a short isoform, interacts with PAK to regulate migration, suggesting that alternative splicing of DPYSL3 may an important to the control of migration. Furthermore Snail is also negatively regulated DPYSL3 with elevated expression in DYPSL3- cells.

In conclusion, the study of the kinome interactome in breast cancer PDX identified DPYSL3 as regulator of the PAK family of kinases in a claudin low breast cancer. DPYSL3 is necessary for PAK to promote migration and may play a pivotal role in complex cellular transitions from a proliferative state versus an EMT/cell migratory state.
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Title: BMP4 suppresses the progression of breast cancer through altered expression of metastasis regulating genes

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Body: Metastasis is a lethal manifestation of cancer, the development of which is the major cause of death in cancer patients. During a search for metastasis-regulating elements, an inverse correlation was identified between the in vivo tumor expression of bone morphogenetic protein-4 (BMP4) and spontaneous metastasis in a panel of isogenic mammary tumors of varying metastatic capacity. BMP4 is an essential morphogen in development, regulating cellular mechanisms akin to those in metastasis, including cellular differentiation, pluripotency and apoptosis. We therefore initiated an investigation of the impact of BMP4 expression on the metastatic process.

We studied the effect of enforced expression of BMP4 in a highly metastatic mammary tumour model called 4T1.2, comparing in vitro properties and tumour progression in mice. There were no differences in proliferation in vitro or when implanted into the mammary gland of immunocompetent mice. In contrast, mice bearing equivalent-sized 4T1.2-BMP4 tumors revealed dramatically reduced metastasis to lung, lymph node and bone. In a parallel study where the established orthotopic primary tumor was resected, survival was significantly extended in mice bearing 4T1.2-BMP4 tumors. Enforced BMP4 expression in tumor cells introduced intravenously resulted in a 2.5-fold decrease in lung metastatic burden, consistent with the impaired capacity of tumor cells to survive in circulation and colonize the lung. Conversely, silencing BMP4 expression in separate weakly metastatic tumours enhanced their ability to colonize the lung and shortened the survival of the mice. No changes were found in the ability of tumor cells expressing BMP4 or treated with recombinant BMP4 to migrate or invade through Matrigel in chemotactic assays but BMP4 enhanced anoikis in both mouse and human breast cancer cells, indicating that BMP4 sensitizes disseminated cells to anoikic stresses induced by cell-substrate detachment and shear flow during systemic transit. BMP4 activated canonical BMP-SMAD signaling in our mammary tumours, leading to altered expression of known metastasis-regulating genes, including SMAD7. SMAD7 depletion in metastasis-deficient 4T1.2-BMP4 tumors accelerated the onset of metastatic disease.

In a meta-analysis of 3,587 breast cancer patients in publically available datasets, low BMP4 mRNA expression was significantly associated with reduced relapse-free survival (RFS) (HR = 0.85, P = 0.01). In an independent analysis using the BreastMark algorithm, low levels of BMP4 mRNA were associated with reduced RFS (HR = 0.88, P = 0.035), distant metastasis-free survival (HR = 0.83, P = 0.035) and overall survival (HR = 0.78, P = 0.006). At the protein level, in a tissue microarray from 415 treatment naïve patients, improved overall survival was observed in multivariate analysis for both BMP4 (HR = 0.66, P = 0.037) and SMAD7 expression (HR = 0.64, P = 0.035) individually. Expression of both proteins compared to neither further improved OS (HR = 0.55, P = 0.005).

In summary, we found strong evidence that BMP4 is a metastasis suppressor correlating inversely with metastatic potential in preclinical breast cancer models and predicting improved relapse-free and overall survival in breast cancer patients.
Title: Expression of adipocyte fatty acid binding protein promotes obesity-associated mammary tumor growth

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Body: Background: Obesity increases the risk of many types of cancer, including breast cancer. The underlying mechanisms that drive obesity-related breast cancer remain unclear. Fatty Acid Binding Proteins (FABPs) are a family of cytosolic proteins which are organ specific and coordinate lipid transportation. Adipocyte FABP (A-FABP) is highly expressed in adipose tissues and may provide a link to obesity-associated breast cancer risk. We previously observed that A-FABP serum levels were associated with obesity, and decreased with surgically induced weight loss. In this report, we evaluate the role of A-FABP in promoting obesity-associated mammary tumor growth.

Hypothesis: Deficiency of A-FABP in mice decreases obesity-associated mammary tumor progression.

Methods: Weaned wild type control and A-FABP-deficient (genetically depleted A-FABP/-) female mice (n=15/group) were randomly grouped and fed either a high fat diet (HFD) (60% fat) or a low fat diet (LFD) (10% fat). After 6 months on the diets, serum was collected from the tail vein and measured for levels of circulating A-FABP by ELISA. E0771 mammary tumor cells (0.5x10^6/mouse) were injected into the mammary fat pad and tumor volume measured at 3-day intervals. The student's t-test was used to compare A-FABP levels and ANOVA to compare tumor sizes in the lean and obese mice.

Results: HFD-fed control mice exhibited a significant increase in body weight (31.5±2.13g) compared to LFD-fed control mice (19.86±0.36) (p<0.01). Serum levels of A-FABP were correspondingly elevated in obese (104.9±8.3 ng/ml) vs. lean controls (31.8±5.4 ng/ml) (p<0.01). Interestingly, although A-FABP deficient mice fed a HFD were more obese than HFD-fed control mice (final body weight 43.4g vs. 31.5g) (p<0.05), tumor growth in the A-FABP deficient mice was significantly less than in controls (p<0.0001), suggesting that A-FABP expression is critical for obesity-associated mammary tumor growth. Long term tumor growth in lean A-FABP deficient vs. control mice was not significantly different. Thus, A-FABP deficiency appears to uncouple obesity and mammary tumor growth in HFD-induced obese mice. Impact: A-FABP is associated with obesity related breast/mammary cancer, and appears to be an important driver of tumor development. Downregulation of this protein may be useful to prevent and/or treat these tumors.
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**Title:** The small Rho GTPase Rho A activation mediates the inhibitory effect of microRNA-200b on triple negative breast cancer cell migration and tumor metastasis

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**Body:** The small Rho GTPases are master regulators of cellular actin cytoskeleton reorganization and focal adhesion dynamic turnover. Many studies including ours have shown that active Rho GTPases promote cancer cell survival, proliferation, migration and tumor metastasis. Therefore, Rho guanine nucleotide exchange factors (Rho-GEFs), positive regulators of Rho GTPases, are generally thought having oncogenic functions; Rho GTPase-activating proteins (Rho-GAPs), negative regulators of Rho GTPases, are usually considered having tumor suppressive functions. Triple negative breast cancer (TNBC) refers to a group of highly heterogeneous malignant tumors that lack estrogen receptor and progesterone receptor expression, and human epidermal growth factor receptor 2 amplification, accounting for 10-20% of newly diagnosed breast cancer cases. Compared to other subtypes of breast cancer, TNBCs usually show worse clinical features such as rapid tumor growth, earlier recurrence, and more aggressive metastasis. Unfortunately, the mechanism of TNBC metastatic behavior has not been well understood. In this study, we investigated the effect of miR-200b, one member of the miR-200 family, on TNBC metastasis using cell culture and mouse orthotopic mammary xenograft tumor models. We found that the expression level of miR-200b is significantly lower in TNBC cells and tissues than that in other types of breast cancer. Stably expressing miR-200b significantly reduced TNBC cell migration and invasion and suppressed TNBC metastasis in a mouse orthotopic mammary xenograft tumor model. Mechanistic studies revealed that miR-200b overexpression in TNBC cells caused drastic changes in cellular actin cytoskeleton organization patterns as evidenced by reduced lamellipodia formation but increased stress fiber formation. In consistent with these findings, Rho GTPase pulldown assays demonstrated that stably expressing miR-200b significantly increased the Rho GTPase Rho A activation, but reduced the Rho GTPase Rac1 activation. Moreover, inhibition of Rho A signaling impaired the inhibitory effect of miR-200b on TNBC cell migration. Bioinformatics analysis indicated that ARHGAP18, a specific Rho-GAP, is a predicated target of miR-200b. Further Q-PCR, Western blot and 3’UTR reporter analysis confirmed that ARHGAP18 is a target of miR-200b. Knocking down ARHGAP18 in TNBC cells using siRNAs significantly increased Rho A activation but reduced Rac1 activation. To further determine the role of ARHGAP18 in TNBC, ARHGAP18 knockout TNBC cells were generated using the CRISPR technology. It was found that knockout ARHGAP18 photocopied the effect of miR-200b overexpression. Moreover, overexpressing ARHGAP18 in miR-200b stable expression cells overcome the inhibitory effect of miR-200b on TNBC metastasis. This study identifies a new mechanism by which miR-200b suppresses TNBC cell migration and tumor metastasis. Moreover, it also demonstrates that ARHGAP18, a Rho-GAP, is essential for TNBC cell growth, migration, tumor growth and metastasis, opposing current dominant view of tumor suppressive roles of Rho-GAPs.
Title: Expression of autophagy related genes impacts clinical outcomes of human breast carcinoma and is associated with estrogen and progestin receptor status

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Body: The dual role of autophagy in breast cancer initiation, progression and responsiveness to various therapies is the focus of extensive studies. Our goal is to assess the relationship of expression of certain autophagy related genes in primary breast carcinomas to predict risk of recurrence. Our hypothesis includes the caveat that unique gene expression subsets will be deciphered by utilizing Laser Capture Microdissected breast carcinoma cells from tissue biopsies containing many cell types.

Methods: Comprehensive analyses of de-identified biomarker results, characteristics of 247 breast carcinoma specimens and clinical outcomes were performed by univariable Cox regressions, Kaplan Meier plots and LASSO with R software version 3.2.5. Microarray analyses were performed on RNA isolated from LCM-procured carcinoma cells to identify gene signatures associated with breast cancer behavior.

Results: Expression levels of 22 autophagy related genes analyzed by univariable Cox regression revealed that RB1CC1, KEAP1, ATG7, RUBCN, NOD2, HMOX1, BECN1, PIK3R4 were significant for predicting Progression Free Survival (PFS) at the discovery level of the adjusted p-value < 0.3. Of these, RB1CC1, KEAP1, & ATG7 were significant for Overall Survival (OS) without regard to ER/PR status. Using Kaplan Meier analyses, expression levels of each of 10 candidate genes predicted PFS of which expression of 6 of these genes also predicted OS using a median split cutoff without regard to ER/PR status. Applying LASSO computations without regard to ER/PR status of the primary breast cancer, a clinically relevant gene expression profile consisting of ATG7, BCL2, HMOX1, KEAP1, NOD2, PTEN, RB1CC1, and ULK4 predicted PFS. Collectively, expression of these 8 genes with TP53 and RUBCN predicted OS without regard to ER/PR status. Violin plots of relative gene expression of each of the 22 candidate genes known to be associated with autophagy pathways revealed that ATG7, ATG12, BCL2 and BECN1 were elevated in ER+ lesions while BCL2 and BECN1 were also elevated in PR+ breast carcinomas. Expression levels of 16 of the 22 autophagy related genes examined by univariable Cox regression were related to either ER or PR status of the primary lesion and predicted either PFS or OS. Refinement of clinically relevant gene subsets was accomplished by LASSO calculations in which either the ER or PR protein status of the primary breast carcinoma was considered. Of the genes in the molecular signatures derived, expression levels in breast carcinomas separated by either ER or PR status of the lesion were tested by Kaplan Meier analyses to assess relationships to PFS and OS.

Conclusions: Using gene expression results derived from microarray analyses of LCM-procured breast carcinoma cells of primary lesions, subsets of autophagy related genes were identified that predict a patient's risk of recurrence and overall survival. Expression of a number of candidate genes appears to be related to either/both the ER or PR protein status of the primary lesion. Collectively, results suggest that expression of certain autophagy related genes may serve as biomarkers for assessing prognosis of breast carcinoma thus impacting clinical management of breast cancer.
Title: Mucin-2 (Muc-2) modulates the biology of breast cancer

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Body: Background: Metastasis and therapeutic resistance are major issues in treating patients with breast cancer. Breast tumors that secrete mucus are generally resistant to chemotherapy. One type of secreted mucin, Mucin-2 (Muc2), is expressed in some breast cancers, but is absent in non-neoplastic breast tissue, suggesting that it plays a role in modulating breast cancer biology. Whether Muc2 expression correlates with breast cancer patient survival is controversial. Thus, the effects of Muc2 on the biology of breast cancer are largely unknown. This study examined the role of Muc2 in modulating breast cancer cell proliferation in vitro and in vivo, response to chemotherapy, and metastasis.

Methods: Two novel cell lines were developed from patient derived tumor xenografts. These two cell lines, BCK4 and PT12, both express high levels of Muc2. In order to modulate Muc2 levels in both cell lines, shRNA targeted to Muc2 (shMuc2) were compared to non-targeting control shRNA (shNT). Decreased expression of Muc2 was confirmed using immunoblotting. Proliferation in vitro was measured using the Incucyte live cell imaging system and crystal violet staining. Response to chemotherapy was measured by examining apoptosis using cleaved-caspase 3 expression. BCK4 and PT12 cells with shNT or shMuc2 were grown as solid tumors in immunocompromised mice and tumor volume measured by caliper. BCK4 cells with high Muc2 (shNT) and low Muc2 (shMuc2) were labeled with luciferase and examined in an experimental metastasis model where total disease was monitored by IVIS imaging.

Results: Decreased Muc2 expression decreased proliferation in BCK4 and PT12 cells versus non-targeting control cells both in vitro and in vivo. Treatment with the chemotherapeutic Docetaxel induced minimal apoptosis in BCK4 control cells with high Muc2 however, apoptosis was significantly increased in BCK4 cells with reduced Muc2. In an experimental metastasis model, mice injected with BCK4 cells containing low Muc2 had decreased disease burden versus those injected with control cells with high Muc2. Endogenous Muc2 expression in wild-type BCK4 cells increased with addition of EGF and this effect was abolished by addition of the EGF-receptor inhibitor, Erlotinib.

Conclusions: Muc2 expression plays an important role in mediating proliferation, apoptosis and metastasis of breast cancer cells. These data suggest that Muc2 is important in controlling the biology of Muc2 positive breast tumors. In addition, Muc2 may be important in guiding treatment and predicting outcomes in breast cancer patients.
The hemidesmosome protein collagen 17A1 is required for collective invasion and growth of mammary tumor organoids

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**Background:** Invasion is a fundamental step in metastasis, the leading cause of breast cancer-related death. We have shown previously that primary breast tumors invade collectively as clusters of cancer cells (Cheung et al. Cell 2013). These clusters are led by a small subpopulation of cancer cells that highly express the basal epithelial protein cytokeratin 14 (K14) and other stem cell genes (Cheung et al. PNAS 2016). K14+ leader cells metastasize as clusters, reverting to a proliferative growth state upon colonization of a secondary site (Cheung and Ewald. Science 2016). The mechanisms regulating their transitions between collective invasion and growth states remains poorly understood. Understanding these mechanisms could produce new insights into tumor cell collectivity and uncover new ways to treat disseminated cell clusters that have already metastasized.

**Results:** RNA-seq analysis of K14+ and K14- tumor cells revealed that K14+ tumor cells have increased expression of genes encoding the hemidesmosome, a major adhesion complex that plays an essential role in anchoring epithelial cells to the basement membrane. The highest upregulated hemidesmosome gene was collagen 17A1 (COL17A1), a transmembrane protein and structural component of the hemidesmosome that is involved in cell migration during wound healing and tissue regeneration. Consistent with these RNA-seq data, we observed by IF staining in vivo that K14 and COL17A1 are typically co-expressed in collective invasive strands at the invasive front of MMTV-PyMT mammary carcinomas.

We next applied ex-vivo 3D culture assays to determine the function of COL17A1 in K14+ cell invasion and proliferation. In mammary tumor organoids we observed that COL17A1 is expressed in basal K14+ tumor cells. However, while the COL17A1 endodomain is uniformly expressed, the ectodomain is expressed in patches, indicating active ectodomain cleavage, a process that reduces cellular motility (Franzke et al. The EMBO Journal 2002). Surprisingly, loss of total COL17A1 expression by shRNA-knockdown significantly impairs tumor organoid growth ~2 fold and prevents invasion in basement membrane rich matrigel. Further studies are underway to investigate the role of COL17A1 during collective invasion in other extracellular matrix environments.

**Conclusions:** Taken together, our data shows that invasive leader cells express COL17A1 in 3D culture and in vivo and that this protein is required for tumor organoid invasion and growth. Therefore, therapeutically targeting COL17A1, a cell surface protein, could prevent both the migratory and proliferative phases of breast cancer metastasis. We are currently investigating how COL17A1 and its shed COL17A1 ectodomain enable leader cells to toggle between invasive and proliferative states.
Title: Investigation of phosphoserine aminotransferase 1 and its role in breast cancer progression

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Body: Metastasis and endocrine resistance are two factors that complicate therapeutic intervention in breast cancer patients and lead to poorer overall survival. Metastasis is known to be responsible for 90% of cancer related deaths, and is especially prevalent in triple negative breast cancer (TNBC); while endocrine resistance can affect up to 50% of patients diagnosed with estrogen receptor positive breast cancer (ER+BC). Phosphoserine aminotransferase 1 (PSAT1) catalyzes the second step within de novo serine biosynthesis and increased expression of enzymes in this pathway have been linked to progression of breast cancer and poor clinical outcomes. Within our preliminary retrospective analysis of human breast cancer patients, we identified an inverse association with elevated transcript levels and poorer distant metastasis free survival, which, coupled to a previously reported correlation of PSAT1 with response to endocrine therapy in patients with ER+BC, we postulate that PSAT1 contributes to breast cancer progression through promotion of metastasis and/or endocrine resistance. To initially determine relevance for PSAT1, immunohistochemistry was performed to determine PSAT1 expression in human breast cancer patients. We found that PSAT1 expression is increased through breast cancer progression, with highest levels observed within metastatic conditions. To investigate the metastatic contribution of PSAT1, we silenced PSAT1 expression within the triple negative breast cancer cell line (TNBC), MDA-MB-231. While suppression of PSAT1 had no effect on proliferation, there was a significant decrease in the motility and invasion of these cells. In addition, decreased PSAT1 substantially inhibited lung metastasis following tail-vein injections of MDA-MB-231 cells in vivo. To investigate PSAT1's role in endocrine resistance, we used parental MCF-7 cells and an endocrine-resistant derivative cell line (LY2) and found that LY2-resistant cells exhibited higher PSAT1 expression compared to parental MCF-7 cells. Lastly, suppression of PSAT1 was able to sensitize the LY2 cells to 4-hydroxytamoxifen treatment. Taken together, these data indicate that PSAT1 may contribute to the progression of human breast cancer via either metastasis or endocrine resistance or both and could potentially serve as a viable target for new therapies.
Title: Detection of disseminated tumor cells in DCIS patients impacts local recurrence

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Body: Background
Recently it was shown that tumor cell dissemination is an early event in breast cancer progression (Hosseini et al., Nature 2016). This has been backed up by an assessment of over 100,000 patients with in situ carcinoma (DCIS), where some patients developed distant metastases and died from breast cancer without presenting with local invasive recurrence (Narod et al., JAMA Oncology 2015). We therefore analyzed the presence and clinical impact of disseminated tumor cells (DTC) in the bone marrow (BM) of DCIS patients.

Methods
BM aspirates were collected from patients undergoing surgery for primary DCIS at Tuebingen University Hospital between 2001 and 2016. Exclusion criteria were presence of invasive breast cancer, bilateral or metastatic disease as well as other malignancies in their history. DTC were identified by immunocytochemistry using the A45-B/B3 anti-pancytokeratin antibody.

Results
627 patients were included. Median follow-up was 49 months. 72 (11%) had detectable DTC. The detection of DTC was not significantly associated with lymph node positivity (3/479, Fisher's exact test: p=0.307) or tumor size (median 30mm, Wilcoxon test: p=0.952).

In 33 (5%) women, a disease recurrence was observed. Of 31 local recurrences 19 were invasive. 7 Patients developed distant metastases, four without an invasive recurrence beforehand. 14 patients died during follow-up.

The detection of at least 2 DTC per 1.5x10⁶ mononuclear BM cells was significantly associated with a lower local recurrence free survival (log-rank: p=0.023) whereas there was no significant difference in distant disease free survival (p=0.315) and overall survival (p=0.083).

Discussion
For the first time, we were able to show that DTC-detection in DCIS patients is associated with local recurrence.

The number of distant metastases in women without invasive local recurrence detected in this cohort was comparable to earlier findings at different centers. This study warrants further investigations concerning evolutionary relationship between primary tumor, minimal residual disease, local recurrence and clinically detectable macrometastases.
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Title: Characterization of novel ESR1 (c.749T>C; p.Met250Thr) mutation in enhancing cellular invasiveness of breast cancer

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Body: Emerging evidence has revealed that the mutations in estrogen receptor alpha (ERα) gene (ESR1) is frequently observed in ER+ metastatic breast cancer, and is associated with the aggressively invasive and metastatic phenotype in advanced breast cancer due to the resistance of endocrine therapy. In our previous study, we have identified three novel mutations of ESR1, including ESR1 G74R, D230H and M250T, in the untreated patients with early breast cancer. However, the functional roles for these novel mutations in the cellular biology of breast cancer remain to be elucidated. In this study, we described the molecular mechanism underlying potential roles for the novel mutation ESR1 p.Met250Thr (c.749T>C) in regulating the cellular invasiveness of breast cancer. Firstly, we found, as compared with wild-type (WT) HA-ESR1, forced expression of HA-ESR1 M250T enhanced the invasive capacity of breast cancer MCF-7 cells by using Transwell assay. Moreover, we found that the levels of miR-190 were significantly up-regulated in the MCF-7 (HA-ESR1 M250T) cells, and further verified that miR-190 played an important role in ESR1 M250T-mediated induction of cellular invasiveness by using specific shRNA to knock down miR-190 levels in MCF-7 (HA-ESR1 M250T) cells. Further bioinformatics analysis showed that there were several half Estrogen Response Elements (EREs) in the promoter region of Talin-2, as the host gene of miR-190. Talin-2-driven luciferase reporter assay indicated ESR1 M250T resulted in a higher increase in the luciferase activity than ESR1 WT. Chromatin-immunoprecipitation (ChIP) assay identified a higher binding ability with Talin-2 promoter for ESR1 M250T than ESR1 WT. Collectively, our mechanistic study revealed that the ESR1 M250T mutation, located in the DNA-binding domain, increased the invasive capacity of breast cancer cells via the transcriptional induction of Talin-2 and miR-190. The potential role for ESR1 M250T in affecting the efficacy of endocrine therapy has been under the investigation in our laboratory, and the result from which will help us better elucidate the clinical relevance for novel ESR1 mutations in affecting the sensitivity of endocrine therapy. This study was supported in part by National Natural Science Foundation of China (8160111571) and Guangdong Natural Science Foundation (2016A030313768).
An increasing number of studies have found that other cells in the tumor microenvironment can influence tumor cells. Adipocytes, which were once thought to function only in energy storage, are now considered an active endocrine organ that secretes pro-inflammatory cytokines and proteases. In human breast cancer patients, invading tumor cells are surrounded by mature adipocytes. The breast cancer cells cause the adipocytes to undergo phenotypic changes and overexpress pro-inflammatory cytokines and matrix metalloproteinases (MMPs), such as MMP-3 and MMP-11. The presence of the changed adipocytes then increases the invasion capacity of breast cancer cells. Obesity is an important breast cancer risk factor. It causes changes to adipocytes and the surrounding microenvironment by invasion of macrophage cells. The effects of obesity-induced macrophage invasion have not been well characterized in the sector of breast cancer-adipocyte crosstalk. A novel 3D co-culture system was used to investigate how macrophage-induced inflammation can worsen this crosstalk, and what changes are involved in cytokine expression, adipocyte de-differentiation, breast cancer aggressiveness, and extracellular matrix (ECM) composition. When macrophage conditioned media was added to adipocytes and breast cancer cells in co-culture, the breast cancer cells showed increased cell proliferation and migration abilities. Experimental co-culture (adipocytes, breast cancer cells, macrophage conditioned media) showed almost twice the number of cells than the control co-culture (adipocytes, breast cancer cells, non-conditioned media). Interestingly, breast cancer cells alone in macrophage conditioned media did not show an increase in proliferation. This shows that macrophages may not affect breast cancer proliferation directly, as all three cell types are needed for increased proliferation of breast cancer cells. Introducing macrophages into this crosstalk also resulted in increased migration abilities. After 24 hours, breast cancer cells grown in experimental co-culture migrated further to close a wound than breast cancer cells grown in control co-culture. These results show that macrophages can affect adipocyte-breast cancer cell crosstalk. This may help explain why obese breast cancer patients have worse prognosis than non-obese breast cancer patients. Further investigation into these effects may identify targets to improve prognosis and abate breast cancer aggressiveness in obese breast cancer patients.
Title: The role of S100A7 in microbiota mediated inflammation and breast cancer progression

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Body: Introduction
While it is accepted that inflammation is a key component of cancer development, the intricate mechanism linking the two are not fully defined. Unparalleled progress has been underway to provide better understanding of this mechanism. We investigate the role of bacterial microbiota in promoting an inflammatory environment through the induction of the pro-inflammatory molecule, S100A7, and its activation of STAT-3 signaling pathways to promote tumor growth and metastasis in breast cancer (BC).

Methods
Immune-competent mouse models of orthotopic breast cancer was used to identify and characterize populations of bacterial microbiota in the cancerous breast tissue at tumor onset. Isolated tissues were homogenized and cultured, then processed for DNA extraction. Bacterial species were identified by aligning the sequences on NCBI BLAST. Mouse mammary tissue and tumors were analyzed for S100a7 after intraperitoneal exposure to LPS after cancer cell injections. Isolated tissues were analyzed by IHC, Western Blot analysis and real time RT-PCR.
mRNA and Protein expression using real time PCR, western blot and flow cytometry, and binding assays analyzed expression and affinity of LPS/S100A7/TLR4 in BC cell lines in vitro under varying conditions. Protein expression and in vitro functional assays including matrigel invasion and wound closure assays determined the effect of LPS/S100A7 on TLR4 and STAT-3 expression and signaling pathways in promoting tumor invasiveness.

Results
We observed microbiota in cancerous breast tissue, which is predominantly composed of Gram-negative bacteria at tumor onset. The population of mainly gram negative bacteria at tumor initiation is unique from those populations from feces and skin. This suggests that breast tissue microbiota may be a potential source of LPS in breast tumors. Stimulation with LPS induces secretion/expression of S100A7 in mouse mammary tissue and tumors, as well as BC cell lines. Furthermore, inhibition of LPS by polymixin B decreases S100A7 to basal levels in BC cell lines. LPS/S100A7 combinational treatment has an additive effect on the invasive potential induced by LPS in BC cell lines as shown by invasion assays and wound closure assays. S100A7 over expression increases TLR4 expression as observed by TLR4 mRNA by real time PCR and protein by flow cytometry. Furthermore, secreted S100A7 protein promotes interaction between S100A7 protein and TLR4 receptor in cell lines with endogenous TLR4 expression. In addition, both S100A7 and LPS stimulation of TLR4 can activate STAT3 signaling pathway, and inhibition of either S100A7 and or TLR4 impairs the invasiveness of BC cell lines.

Conclusion
A unique population of gram negative bacteria characterizes breast cancer tissues. LPS of bacterial cells walls, representative of gram negative bacteria induces S100A7, which interacts with TLR4 to activate the STAT-3 pathway in tumors. This LPS-S100A7-TLR4-STAT3 axis in turn increases the invasiveness of tumor cells to promote tumor metastasis. This suggests that microbiota plays an important role in the initiation and progression of breast cancer through regulation of the pro-inflammatory molecule S100A7.
Title: Adjuvant statin therapy efficacy is dictated by tumor dormancy and statin lipophilicity in ex vivo and in vivo models of metastatic breast cancer

Colin H Beckwitt¹, Amanda M Clark¹, Katsuhiko Warita², Zoltan N Oltvai¹ and Alan Wells¹. ¹University of Pittsburgh School of Medicine and ²Tottori University School of Veterinary Medicine.

Body: Metastasis in breast cancer patients heralds mortality, as disseminated disease is generally chemoresistant. After tumor cells reach the ectopic tissue, they undergo an epithelial reversion to enter a period of quiescence, termed dormancy, which may last for decades before outgrowing again as mesenchymal/dedifferentiated masses. Thus, long-term, relatively non-toxic interventions that prevent metastatic outgrowth are needed to treat this mortal stage of tumor progression. Epidemiological analyses have suggested that statin usage, for cardiovascular indications, is correlated with a reduction in clinically-evident metastatic (though not in incidence of primary) breast cancer. The goal of this study is to demonstrate this is due to statins suppressing breast cancer cell proliferation and keeping the micrometastases in the dormant state. We have found that atorvastatin and simvastatin limit the growth of some cancer cell lines, but not others. The sensitive lines were marked by lacking surface E-cadherin, the hallmark of the mesenchymal phenotype. When E-cadherin is downregulated on epithelial tumor cells, the cells become growth inhibited by the statins. Furthermore, this is a direct effect, as we now have shown that hydrophilic statins are relatively ineffective compared to the membrane permeant lipophilic statins as tumor cells generally lack the transporters that enable these drugs to gain access to the cells.

To determine whether the statins target the emergent metastatic tumor cells, we are using an all human microphysiological system (MPS) of the most common site for metastases, the liver. Briefly, a micro-hepatic tissue is established by seeding primary human liver cells in a porous scaffold subject to a physiological flow. RFP-labeled breast cancer cells are seeded into these microtissues and examined weeks later. Liver function and health are monitored by clinical chemistry assays performed on supernatant samples. We have previously shown that this system robustly reproduces tumor dormancy. Initial studies suggest that statins suppress the emergence of dormant tumor cells when challenged by stressors that lead to outgrowth. Additionally, atorvastatin suppresses proliferation of mesenchymal but not epithelial breast cancer cells in intrasplenic and mammary fat pad injection models for breast cancer metastasis to the liver and lung respectively. As 26% of adults currently take a statin for other medical conditions, these studies may suggest the best statin to use in the context of maintaining breast cancer dormancy long-term and delaying or avoiding the morbid emergence.
The levels of sphingosine-1-phosphate and its related gene expressions in breast cancer patients

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Body: Background: The bioactive lipid mediator sphingosine-1-phosphate (S1P) has emerged as a key regulatory molecule in cancer progression. We previously demonstrated that S1P is a crucial mediator of breast cancer-induced angiogenesis and lymphangiogenesis, and promote metastasis. Although increasing number of in vitro and in vivo experiments have revealed the importance of S1P in cancer progression, the data on the roles of S1P in human patients are very limited. The aim of this study is to reveal the clinical relevance of S1P in the interaction between cancer and the tumor microenvironment by examining the levels of the sphingolipids in patient breast cancer tissue samples.

Material and Method: Breast cancer tissue, peri-tumor tissue, and normal breast tissue were collected from 20 breast cancer patients immediately after surgery that were conducted from November 2015 to February 2016 at Niigata University Medical and Dental Hospital. Sphingolipids were quantified by liquid chromatography–electrospray ionization tandem mass spectrometry. The expression level of each enzyme-encoding gene involved in S1P production was evaluated by retrieving RNA sequencing and gene expression quantification data from breast cancer tissues (n = 112) and paired normal breast tissues (n = 112) using the Genomics Data Commons (GDC) data portal of the The Cancer Genome Atlas (TCGA) cohort. Gene expression levels were derived using normalization methods provided in the DESeq2 package.

Result: The levels of the sphingolipids sphingosine (Sph), dihydro-sphingosine (DHSph), S1P, and dihydro-S1P (DHS1P) were successfully determined in breast cancer, peri-tumor, and normal breast tissues from all of the 20 patients. As expected, a one-way ANOVA revealed that S1P levels were significantly different depending on the location (F(2,57) = 7.029, P = 0.002). The Tukey post hoc test revealed that S1P levels in tumors were significantly higher than those in normal breast tissue and peri-tumor tissue (P < 0.05). Similarly, Sph and DHSph levels in tumors were significantly higher than in normal breast tissue and peri-tumor tissue. Both SPHK1 and SPHK2 gene expression levels in breast cancer tissue were higher than those in normal breast tissue. Interestingly, expression of some of the S1P-related genes; S1PR3, ABCC1, SGPL1, and ORMDL2, were significantly increased in the breast cancer tissue compared to normal breast tissue. On the other hand, there was significantly decreased expression of the S1P-related genes S1PR1, S1PR2, ABCG2, SPNS2, SGPP1 and ORMDL3, in breast cancer tissue compared to normal breast tissue.

Conclusion: We demonstrated that the major source of S1P is the tumor tissue, and not the peri-tumor tissue despite the fact that angiogenesis and lymphangiogenesis are occurring more in the peri-tumor area, which implicate that S1P may have further role inside the tumor. Our results indicated the complexity of S1P signaling in human cancer than expected based on the results in vivo experiments.
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**Title:** TGFβ-Smad3-TMEPAI axis drives the tumor progression of triple negative breast cancer

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**Body:** **Background:** In the absence of mutations and/or deletions in the TGF-β signaling molecules, how triple negative breast cancer (TNBC) cells become resistant to growth inhibitory signaling of TGF-β was addressed by screening of several TNBC cell lines for Smad proteins involved in TGF-β signaling. Unexpectedly, altered Smad2 and Smad3 protein levels were detected in breast cancer cells relative to normal mammary epithelial cells, suggesting a novel mechanism to escape from TGF-β mediated growth inhibition. In order to reflect the true functional status of the endogenous molecules, Smad2 or Smad3 were individually knocked down and tested its effect on pro-oncogenic behavior of TGF-β.

**Materials and Methods:** All cell lines were cultured according to the recommended standard procedures. TMEPAI knockdown was achieved by using lentiviral vectors and knockdown of Smad2 and Smad3 was achieved by using retroviral vectors. DNA transfections and luciferase assays were performed according to vendor instructions. Cell proliferation was measured by quantitation of total cellular DNA. Immunoblotting, invasion and immunohistochemical assays were performed using standard methods. **Bioinformatics:** Triple negative breast cancer patient dataset (GSE58812) was used to compare Smad2, Smad3, Smad4 and TMEPAI/PMEPA1 expressions correlated with overall survival using the PROGgene tool by using median gene expression value as a dividing point.

**Results:** While Smad2 deficiency has no effect on breast cancer cell behavior, Smad3 deficiency reduced growth and invasion capacity of breast cancer cells. Interestingly, Smad3 deficiency was associated with reduced gene expressions of transmembrane prostate androgen induced (TMEPAI or PMEPA1) gene and EMT inducing transcription factors and increased expression of cell cycle inhibitors. In contrast, Smad2 deficiency had opposite effect on these regulators. Importantly, the decreased growth and invasion and their associated gene expressions in Smad3 knockdown cells were largely reversed by overexpression of TMEPAI. Meta-analysis of Jezequel dataset of triple negative breast cancer patients suggested higher TMEPAI/PMEPA1 and lower Smad2 expressions are significantly associated with decreased survival. Our results support the idea that development of several triple negative breast cancers may involve expansion of cell populations with altered Smad2 and Smad3 levels resulting in Smad3 dependent expression of TMEPAI, which provides a competitive advantage for cancer cells to grow and metastasize in presence of TGF-β. Moreover, following meta-analysis of published microarray datasets in lung and gastric cancers also revealed that increased TMEPAI and Smad3 expression and decreased Smad2 expressions were significantly associated with poorer patient prognosis in non-small cell lung adenocarcinomas and gastric cancers suggesting universality of this phenomenon.

**Conclusion:** We identified TGF-β-Smad3-TMEPAI signaling axis as a driver of tumor progression in triple negative breast cancer. Our results suggest that novel therapeutics targeting TMEPAI will selectively inhibit oncogenic activity of TGF-β and promote its tumor suppressive activity in treating TNBC.
Title: Hypoxia induced centrosome amplification via HIF-1α/Plk4 signaling axis associates with poorer overall survival in TNBC

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Body: Background: Centrosome amplification (CA) which refers to presence of supernumerary or abnormally large centrosomes drives tumor progression by promoting chromosomal instability and the generation of aggressive tumor clones. Although the role of CA in cancer progression is well-defined, no studies have yet discussed how CA is induced in tumor cells. We report here that intra-tumoral hypoxia, which is considered one of the major contributors to intratumor heterogeneity, induces CA via HIF-1α.

Methods: We first immunohistochemically labeled 24 breast carcinoma and uninvolved adjacent normal tissue samples for HIF-1α and calculated weighted indices (WIs) for nuclear HIF-1α. Adjacent serial sections from the same tumors were also immunofluorescently labeled for γ-tubulin and CA was calculated. Using public microarray datasets (Kao dataset, n=327), we investigated whether centrosomal gene expression is enriched in breast tumors characterized by a hypoxia gene expression signature. Finally, to determine the role of hypoxia in CA induction we exposed cultured TNBC cells (MDA-MB-231 and MDA-MB-468) to hypoxia and overexpressed (OE) and knocked out (KO) HIF-1α in TNBC cells and quantitated CA. Additionally, to discern the biological pathway through which HIF-1α induces CA we performed ChIP assay and in silico analyses to identify the possible targets of HIF-1α.

Results: A strong positive correlation between nuclear HIF-1α WI and CA was found in breast tumor samples (Spearman's rho p=0.722, p<0.001). In addition, we found that higher nuclear HIF-1α was associated with worse overall survival (p=0.041; HR=1.03). Our in silico findings suggest that breast tumors with high expression of hypoxia-associated genes exhibited higher expression of centrosomal genes than breast tumors with low expression of hypoxia-associated genes. In addition, cells cultured in hypoxic conditions exhibited ~1.5 fold higher (p<0.05) CA when compared to the cells cultured in normoxic conditions. Interestingly level of CA decreased when HIF-1α KO TNBC cells were exposed to hypoxia and it increased when HIF-1α OE TNBC cells were culture in normoxic conditions. Furthermore, we discovered that HIF-1α induced CA by directly regulating the expression of Plk4 which was confirmed by performing ChIP assay. Our results indicated HIF-1α interaction with the motif in the PLK4 promoter from genomic DNA of MDA-MB 231 cells under hypoxic conditions, was significantly (p=0.04) higher when compared with the cells cultured under normoxic conditions. Plk4 mRNA expression was assessed using the online BC gene expression data sets (n=25). We found significantly higher expression of Plk4 in TNBC (n=374) when compared with non-TNBC (n=4098) and it was associated with poor overall survival (HR=1.76; p=0.054) in TNBC.

Conclusion: Collectively our findings suggest that hypoxia drives CA in TNBC via HIF-1α and contribute to poor outcomes. Thus, determination of CA and HIF-1α can help risk stratification in TNBC patients for more personalized treatments.
Body: Background: Combination of metabolomics and epidemiological approaches opens new perspectives for ground-breaking discoveries. The aim of the present study was to investigate for the first time whether plasma non-targeted metabolomic profiles, established from a simple blood draw from healthy women, could contribute to predict the risk of developing breast cancer within the following decade and to better understand the etiology of this complex disease.

Methods: A prospective nested case-control study was set up in the SU.VI.MAX cohort, including 206 breast cancer cases diagnosed during a 13y follow-up, and 396 matched controls. Non-targeted NMR metabolomic profiles were established from baseline plasma samples. Multivariable conditional logistic regression models were computed for each individual NMR variable and for combinations of variables derived by principal component analysis.

Results: Several metabolomic variables from 1D NMR spectroscopy were associated with breast cancer risk. Women characterized by higher fasting plasma levels of valine, lysine, arginine, glutamine, creatine, creatinine, and glucose and lower plasma levels of lipoproteins, lipids, glycoproteins, acetone, glycerol-derived compounds and unsaturated lipids had a higher risk of developing breast cancer. P-values ranged from 0.00007 (OR_{T3vsT1}=0.37[0.23-0.61] for glycerol-derived compounds) to 0.04 (OR_{T3vsT1}=1.61[1.02-2.55] for glutamine).

Conclusion: This study highlighted associations between baseline NMR plasma metabolomic signatures and long-term breast cancer risk. These results provide interesting insights to better understand complex mechanisms involved in breast carcinogenesis and evoke plasma metabolic disorders favorable for carcinogenesis initiation. This study may contribute to develop screening strategies for the identification of at-risk women for breast cancer well before symptoms appear.
Title: FASN inhibition by TVB-3166 associates with breast cancer subtype

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Body: Fatty acid synthase (FASN) is overexpressed in numerous tumor types, including breast carcinomas, and promotes changes in the genetic program controlling lipid biosynthesis. While inhibiting FASN appears to be an attractive therapeutic approach under development, the success of this approach may depend on the identification of tumor subtypes with specific metabolic requirements. Applying a comprehensive profile of circulating tumor cells (CTC) using canonical pathway gene sets, we identified a correlation of metabolic subtypes with breast tumor subtype. A lipogenic subtype is strongly associated with Luminal A subtype, whereas the glycolytic subtype associated with Luminal B tumors. The triple negative subtype was more heterogeneous and had the expression of both sets of gene. Such a difference in the metabolic profile may dictate differential sensitivity to inhibitors targeting de novo lipid synthesis, including FASN. This was supported by in vitro studies using selective FASN inhibitor, TVB-3166. Exposure to TVB-3166 over 14 days incubation in Advanced MEM with 1% charcoal-stripped FBS selectively inhibited growth and viability of Luminal A breast cancer cells, but had no effect on Luminal B subtype. This was further confirmed in short-term patient derived cultures. Mechanistic studies suggest that TVB-3166 quickly disrupts FA synthesis leading to the disruption of the lipid raft architecture and tumor cell death through an apoptotic mechanism. In conclusion, our findings highlight that success of targeting cancer metabolism directly may depend on identification of tumor subtypes with specific metabolic requirements.
Title: The Y537S ESR1 mutation carries unique metabolomics profiling in breast cancer

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Body: Background:
Estrogen receptor (ESR1) mutations occur at a high frequency in metastatic breast tumors in patients treated with hormonal therapy in the metastatic setting. We do not know if these mutations changed metabolomics and whether these metabolomics change could affect metastasis.

Experimental design and methods:
We generated ESR1 Y537S homozygous mutations using CRISPR Casp-9 technology. Globe metabolites screening was performed using 6550 Agilent QTOF instrument. Athymic mice were used in tumor xenograft studies. Affymetrix microarrays were performed to compare gene expression changes in Y537S mutant compared with parental cells. Enriched metabolite pathways and gene expression integrated analysis was analyzed by using online analysis tool http://www.metaboanalyst.ca.

Results:
We generated CRISPR ESR1 Y537S mutation homozygous knock-in clones in MCF-7 cells. In vivo experiments revealed that mutant cells are dominant drivers of metastasis. Transcriptome profiling revealed elevated expression of Hallmark pathways, including EMT and estrogen-regulated gene expression. We performed globe metabolites screening using MCF-7 Y537S and MCF-7 parental and identified 134 metabolites. Serum starvation media was used and estrogen was used as control for both cell lines. As we expected estrogen treatment induced metabolites changes in parental cells. However, metabolites in mutant cells were not changed significantly under estrogen treatment. Interestingly, metabolites in the mutant cells at baseline were remarkably upregulated (78 out of 134 identified total metabolites) indicating mutant cells in serum starvation condition had significantly different metabolomics compared with parental cells. Top upregulated pathways include protein biosynthesis, betaine metabolism and ammonia recycling. Integration of microarray gene expression and metabolites reviewed several metabolomics pathways significantly changed in mutant compared with parental cells including for example aminoacyl-tRNA biosynthesis, arginine and proline metabolism and alanine, aspartate and glutamate metabolism.

Conclusion: The Y537S ER mutation is a driver of distant metastasis in ER-positive breast cancer cells. Y537S ER mutant had globe changes of metabolites expression which was confirmed by integrated analysis combining microarray gene expression. The roles of these metabolites need to be studied to correlate with metastasis. Enzymes responsible for converting these metabolites changes could be served as potential therapeutic targets.
Metabolomics shows distinct pattern of one-carbon metabolism in invasive breast cancer

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Body: One-carbon metabolism involving the folate and methionine cycles integrates nutritional status from amino acids, glucose and vitamins, and generates diverse outputs, such as the lipidic nucleotide and proteic biosynthesis and the maintenance of redox status and substrates for methylation process. Long considered as a 'housekeeping' process, this pathway has recently been shown additional complexity. Genetic and functional evidence suggest that one-carbon metabolism hyper activation works as oncogenesis driver and establishes a link to cellular epigenetic status. However, results in breast cancer area remain inconclusive. Methods: In this sense, target metabolomic analysis was performed from primary BC and adjacent tissue samples from a cohort of 182 breast cancer patients treated at the Institut Gustave-Roussy and Institute Curie – Paris, University of Debrecen – Hungary, and University of Tartu – Estonia. The Biocrates Life Science® company performed the metabolomics analysis using the Absolute IDQ p180 kit. Statistical analysis was performed by MetaboAnalyst 3.0 software (p <0.05). We performed the combined analysis of genes from public database (GEO: GSE61725) with genes of interest related to one-carbon metabolism. Differently expressed genes analysis between breast tumor and normal breast tissue were performed by TMev program using test T student (p≤0.05). We selected only differently expressed genes related with one-carbon metabolism.

Results: We observed that metabolites of one-carbon such as glucose, serine and threonine, precursors of glycine, as well as, glutamate, glutamine and production of glutathione increased when compared with adjacent tissue samples. Also, we found a rising of methionine, assymetric dimethylated arginine (ADMA), and arginine used in redox homeostasis, in protein post-translational modification and epigenetic events. Surprisingly, the leucine metabolites indicated a link to cholesterol synthesis. In tumor tissue samples an increase of saturated fatty acid synthesis (SFA) was observed, such as palmitic acid, sphenolipids and phosphatidylycolines SFA enriched when compared to normal tissue, suggesting an increase of lipid rafts and membranes in the breast tumor cells. To validate these data, we found 11 genes differently expressed genes related to one-carbon metabolism from public DataBase: G6PD, GLUT4, GLS, STK11, PRMT1, NOX1, AUH, ABCG1, HMGCS2, DHCR7 and NPC1. Conclusion: Our results indicate that one-carbon metabolism were hiperactivated in primary breast cancer when compared with adjacent tissue. Given the wealth of clinically available agents that target one-carbon metabolism, these new findings could present opportunities for treatment in breast cancer medicine.
The effect of obesity and metabolic factors on genomic assays for risk of recurrence

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**Body: Background:** Obesity and other factors contributing to metabolic syndrome (MS) have become increasingly prolific in today's population. They are also known risk factors or comorbidities in many disease states including breast cancer (BC). While obesity and MS are known to increase the risk of developing BC, the effect on the risk of distant recurrence is uncertain. This substudy evaluated the relationship of BMI, blood pressure, cholesterol, triglyceride, diabetic status, and MS on the risk of recurrence in early BC patients. **Methods:** The effect of these metabolic factors on MammaPrint (MP) and Oncotype DX (ODX) risk of recurrence assays was compared. Additionally, gene expression analysis was performed to elucidate the differences between obese patients who were MP high risk (HR) vs. low risk (LR), and MP HR patients who were obese vs. not-obese. This subanalysis included patients from the PROMIS (n=198) and IMPACt (n=382) studies for whom metabolic characteristics were captured with informed consent. From the IMPACt study, 98 patients also reported ODX results (RS range 0-69). **Results:** By BMI, 1% of patients were classified as underweight (BMI <18.5), 22% normal (18.5 ≤ BMI <25), 31% overweight (25 ≤ BMI <30), and 46% as obese (BMI ≥30). To be classified as having MS, the patient had to exhibit any 3 of the 5 metabolic factors. In the full cohort, having MS was significantly associated with ethnicity (p < 0.05) and menopausal status (p < 0.001), but not histopathological tumor type, histological grade, tumor stage, or lymph node status (p > 0.05). 36% (207/580) of patients were considered as having MS in the full cohort, similarly 36% (35/98) had MS in the ODX subset. Of the patients with MS, MP classified 52% (108/207) as HR. For the subset of ER-positive patients with an ODX result, ODX classified the 11% (4/35) as HR whereas MP classified 57% (20/35) of the same patients as HR. **Conclusions:** Patients with obesity as measured by BMI and with MS were more frequently classified as HR by MP, in contrast to ODX which classified nearly all patients as intermediate or LR, confirming previous findings by Robinson, et. al (SABCS 2012 and 2014). Understanding the biological foundation of how obesity and metabolic factors affect risk of recurrence in breast cancer will improve both the treatment and care of patients.
Body: Background
Reprogramming of metabolism is a hallmark in cancer. In previous works we observed differences in glucose metabolism between tumors from different breast cancer subtypes, suggesting the possibility to use drugs against metabolism in this disease. Flux Balance Analysis (FBA) is widely used to study biochemical networks, allowing to predict growth rates and to simulate drug response.

Material and methods
Breast cancer cell lines and different drugs against metabolic targets were evaluated with dose-response curves, and pharmacological parameters for each condition were calculated. Proteomics data from breast cancer cells lines treated with sub-lethal doses and controls were obtained applying a mass spectrometry-based approach. Differences in protein expression between treated vs. control were assessed. An FBA approach using the human metabolic reconstruction Recon2 and including the protein expression values from perturbation experiments was also applied. Model predictions were validated using dynamic FBA and growth rate for each sample was estimated. With the aim to compare the activity of the different pathway fluxes between control and treated cells, flux activity was calculated for each condition and for each pathway and response predictive models were performed.

Results
Drug response was diverse across different breast cancer cells. Mass spectrometry from cell samples allows identifying and quantifying 4,114 proteins. FBA predicted that growth rates decrease in treated cells vs. control, as observed in cell viability assays. Dynamic FBA showed that our model correctly reflects cell growth rates. Finally, using flux activities, it is possible to build models which could predict response against these drugs.

Conclusions
Proteomics provide insights of the mechanisms responsible of cells' response to metabolism drugs. A validated computational model able to predict tumor growth using data from proteomics was developed. Model predicts growth rates and also dysregulation of biological processes triggered by drug treatment. Moreover, these computational approaches could be used to propose new mechanisms of action and effects of metabolic drugs.

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Competing interest
JAFV, AG-P and EE are stakeholders of Biomedica Molecular Medicine S.L. and Biomedica Molecular Medicine Ltd. LT-F is an employee of Biomedica Molecular Medicine S.L. The authors have declared no other conflict of interest.
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**Body:** Background - The metabolic syndrome (MetS) is prevalent among post-menopausal breast cancer patients and is associated with increased breast cancer risk. Mammographic breast density (BD) is also positively associated with increased breast cancer risk. The relationship between MetS and mammographic BD is unclear and requires further investigation.

**Aim** - The aim of this study was to examine the relationship between the MetS and its component features with breast density.

**Methods** - 112 post-menopausal women with breast cancer were recruited. Body composition (Body Mass Index (BMI), waist circumference (WC)) was measured objectively in participants prior to surgery. Metabolic profiles were measured in blood taken from participants prior to surgery. MetS was defined according to the International Diabetes Federation (IDF) criteria. BD was classified according to the Breast Imaging Reporting and Data System (BI-RADS). Participants were categorised into those with 'Dense' (BI-RADS score 3 or 4) or 'Less Dense' (BI-RADS score 1 or 2) breasts. Group means were compared using unpaired t-tests for parametric or Mann Whitney tests for non-parametric data. Categorical data was analysed using Fisher's exact test or Chi squared test as appropriate.

**Results** - An inverse relationship was observed between measures of adiposity and BD. Participants with 'dense' (BI-RADS 3/4) breasts had significantly lower BMI ($p=0.0034$), waist circumference ($p=0.0007$), systolic blood pressure ($p=0.03$), circulating insulin level ($p=0.009$) and glycated haemoglobin ($p=0.008$) than those with 'less dense' (BI-RADS 1/2) breasts. HDL was significantly higher in those with 'dense' versus those with 'less dense' breasts ($p=0.03$). Participants with 'less dense' breasts were significantly more likely to be insulin resistant (HOMA-IR $\geq 2$) than those with 'dense breasts' (50.6% versus 20% respectively); $p=0.01$.

Other components of the MetS (Serum triglycerides, glucose and diastolic blood pressure) did not differ significantly between participants with 'dense' and 'less dense' breasts. No differences in overall survival were observed between participants with 'Dense' versus those with 'Less Dense' breasts ($P=0.93$).

**Conclusion** - Although both MetS and BD are positively associated with breast cancer risk; it is unlikely that the MetS is related to an increase in breast cancer risk through a mechanism involving BD. Further work on this study is currently underway and will involve adjusting for potential confounders including age and BMI as well as examining the relationship between MetS and BD in pre-menopausal breast cancer patients.
Reprogramming glucose metabolism and energy production in breast cancer cells

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Most mammalian cells use glucose as a main fuel source. Glucose is metabolized via glycolysis to pyruvate, which enters the mitochondria and then generates ATP through Krebs cycle in normal condition. However, metabolism is characteristically reprogrammed and cancer cells or highly proliferative cells preferably generate ATP through lactate production by lactate dehydrogenase (LDH/LDHA), referred to as the Warburg effect or metabolic reprogramming toward aerobic glycolysis. Efficient control of energy metabolism is the key to maintaining metabolic homeostasis, and disturbance in energy balance provokes diseases such as obesity, diabetes and cancer. However, the mechanisms underlying efficient energy metabolic homeostasis and breast cancer development are poorly understood. HJC0152, a novel small molecule glucose metabolism modulator, was developed using structure- and fragment-based drug design strategies and molecular modeling techniques. Aggressively growing and metastatic breast cancer cells of triple-negative subtype (MDA-MB-231) treated with HJC0152 showed decreased activity and protein level of LDHA, which resulted in a decrease lactate production. In addition, these cells also exhibited decreased glucose uptake and HK2 protein level. Furthermore, the amount of intracellular ATP in MDA-MB-231 cells was significantly reduced. Our findings suggest that HJC0152 is capable of reprogramming cancer metabolism by modulating glucose metabolism and ATP production. These results may provide a rationale to develop HJC0152 as an effective therapeutic for cancer and other metabolic diseases with aberrant glucose metabolism. In addition, HJC0152 can serve as a molecular probing tool for elucidating the key factors responsible for developing breast cancer and other metabolic diseases. This work was supported by Grants P50 CA097007, and P30DA028821 (JZ) from the NIH, CPRIT (JZ), John Sealy Memorial Endowment Fund (JZ), DFI Grants from MD Anderson Cancer Center (QS), Holden Family Research Grant in BC Prevention (QS), and NCI PREVENT Program HHSN26100002 (QS).
Targeting breast cancer stem cell state equilibrium through modulation of redox signaling

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Body: Breast cancer stem cells (BCSCs) maintain the plasticity to transition between quiescent mesenchymal- (M) and proliferative epithelial-like (E) states, but how this plasticity is regulated under metabolic/oxidative stress is poorly understood. Here, we show that M- and E-BCSCs exhibit markedly different sensitivities to the inhibitors of glycolysis and redox metabolism. Metabolic/Oxidative stress generated by 2DG/H₂O₂ or hypoxia promotes ROS⁻ M-BCSCs transition to their ROS⁺ E-state. This transition is reversed by the antioxidant N-acetyl cysteine and facilitated by the activation of the AMPK-HIF1α axis. Moreover, E-BCSCs exhibit robust expression of NRF2/NFE2L2 and a wide variety of NRF2 downstream antioxidant responsive genes including the family of drug transporters and detoxification enzymes, NADPH production as well as the thioredoxin (TXN) and glutathione (GSH) antioxidant pathways. Suppression of NRF2 activity by a small-molecular inhibitor Trigonelline or shNRF2 mediated knockdown significantly decreased ALDH⁺ E- but not CD24⁻CD44⁺ M-BCSCs. This specific vulnerability of E-BCSCs to the inhibition of NRF2-mediated antioxidant defenses was also observed following inhibition of the downstream TXN and GSH antioxidant pathways, which promotes ROS-mediated differentiation and subsequent apoptosis of E-BCSCs. Co-inhibition of glycolysis and TXN/GSH pathways synergistically suppressed tumor growth and tumor initiating potential in two patient-derived xenograft models of triple negative breast cancer by eliminating both M- and E-BCSCs. Together, our studies reveal novel cellular and molecular mechanisms demonstrating how modulation of redox signaling regulates the equilibrium of two distinct BCSC states. These studies define the metabolic vulnerabilities of M- and E-BCSCs, and also provide a novel therapeutic approach to collectively target these distinct CSC states. As the CSC state equilibrium may be similarly regulated across a spectrum of tumors with diverse oncogenic drivers, this approach may have broad therapeutic applicability.
Title: Preclinical efficacy of a novel and potent inhibitor of the MDM2-p53 axis AMG 232 in ER+ breast cancer model

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Body: Background: Approximately 70% of breast cancers (BC) express estrogen receptors (ER) and/or progesterone receptors (PR) which define their degrees of estrogen dependence. Although mutations in the tumor suppressor TP53 gene are thought to be the most abundant genetic alterations occurring in cancers, the relative prevalence of TP53 mutations in ER+ BC is low (luminal A 12% and luminal B 29%) compared to HER2+ or TNBC, and total MDM2/4 alterations are about 12% (Cancer Genome Atlas Network Nature. 2012). In our (Avera Cancer Institute, Sioux Falls, SD) ER+ BC cohort TP53 and MDM2/4 alterations are 25% and 14% respectively. Purpose: Blocking the interaction between MDM2 and p53 may generate a novel treatment opportunity in TP53 wild-type ER+ BC patients. Methods: Comprehensive genomic profiles from 160 ER+ BC patients (February 2014 through May 2017) were analyzed. Patients were biopsied after consultation and samples were analyzed for genomic [FoundationOne] and proteomic analyses [Theranostics]). We also evaluated mutation distribution in cell-free DNA via digital NGS using the Guardant360 panel. We tested the anti-tumor efficacy of MDM2 inhibitor (AMG 232) alone or a combination of an aromatase inhibitor, letrozole plus AMG 232 in ER+ BC model (MCF7, Zr-75-1, & MDA-MB415). Results: 1) AMG 232 binds the MDM2 protein, blocks the MDM2-p53 interaction and induces p53 expression and activity, 2) p53 effector molecule p21 is robustly induced following the treatment of AMG 232, 3) the anti-proliferative activity of AMG 232 was observed by 3D-ON-TOP clonogenic assay and real-time monitoring in an IncuCyte Zoom, 4) ER+/TP53 wild-type (WT) cell lines exhibited an increase in annexin V positivity (initiation of apoptotic activity) following AMG 232 treatment. AMG 232 also induced the apoptotic markers cleaved-CASPASE3 and cleaved PARP1 protein expression in ER+/PR+/TP53 WT and PTEN null breast cancer cell lines, 5) AMG 232 treatment induced G1 cells cycle arrest (Flow cytometric analysis of PI staining) dose dependently which may be associated with upregulation of p21. 6) we also assessed mRNA expression (several pro-apoptotic and anti-apoptotic molecules) by RT-qPCR following the treatment of AMG232 in MCF7 (ER+/TP53 WT) and Zr-75-1 (ER+/PR+/TP53 WT and PTEN null) cell lines. Similar to our protein expression data, mRNA data also showed that pro-apoptotic/cell cycle inhibitor transcripts such as p21 and PUMA mRNA expression were significantly increased following the treatment of AMG 232. On the contrary anti-apoptotic/ pro-survival transcripts such as survivin (BIRC5) and stathmin (STMN1) mRNA expression were significantly decreased following the treatment of AMG 232 and 7) importantly, normoxic-proteasomal degradation of HIF1α (responsible for the development of chemotherapy and targeted therapy resistance) was not rescued by prior treatment of this novel MDM2-p53 interacting inhibitor AMG 232 in Zr-75-1 cell line. Conclusion: Taken together, our data suggest that AMG 232 effectively inhibited proliferation and enhanced apoptosis via inhibition of the MDM2-p53 interaction and p53-mediated downstream signaling events in ER+/TP53 wild-type BC model.
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Title: Invasive lobular carcinoma and invasive ductal carcinoma differ in immune response, translation efficiency and metabolic rate

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Body: Background

Invasive lobular carcinoma (ILC) is the second most common histological subtype of breast cancer after invasive ductal carcinoma (IDC). ILC differs from IDC in pathologic, molecular, and clinical features. ILC tumors are most often characterized as luminal A by PAM50 analysis, suggestive of an indolent disease. Yet, when matched for receptor status and tumor grade, patients with ILC tend to have worse long-term outcomes than patients with IDC. The main distinguishing molecular feature of ILC is the loss of functional E-cadherin, and yet, beyond that loss, the mechanisms underlying the differences between ILC and IDC are largely unknown. We examined the RNA expression profiles of ILC and IDC tumors to assess if there may be underlying vulnerabilities of ILC tumors to novel therapeutic strategies.

Methods

Differential expression analysis was performed on 159 luminal A (LumA) ILC tumors versus 311 LumA IDC tumors from The Cancer Genome Atlas (TCGA). The METABRIC cohort (65 LumA ILC and 533 LumA IDC) was used as a validation dataset. Pathway enrichment analysis was performed to identify potential differences in biological processes, and these potential differences were then tested in a series of in vitro experiments, using 3 ER+ ILC (MDA-MB-134VI, SUM44PE, and MDA-MB-330) and 3 ER+ IDC (MCF7, T47D, and ZR75.1) cell lines.

Results

Pathway analysis led to the identification of three main signaling differences between LumA ILC and LumA IDC: immune regulation, translation, and metabolism. A series of immune pathways, including Immunological Synapse, Biocarta IL17 pathway, and Response to Wounding were up-regulated in ILC tumors. We examined specific cell type markers, and found that ILC tumors have a higher activity of nearly all immune cell types, including CD4+ T cells, CD8+ T cells, B cells, NK cells, dendritic cells, M1 macrophages, and M2 macrophages. These results were surprising, since ILC tumors have a lower incidence of stromal inflammation, as defined by H&E staining, suggesting a unique immune regulatory mechanism in ILC.

Next, we examined the translational regulation in ILC vs IDC tumors by comparing RNA expression and protein quantities as determined by RPPA analysis. ILC tumors have a lower protein:RNA ratio, suggesting a lower translation efficiency. This was reflected in the RPPA data by lower protein expression of eIF4G, ribosome protein S6 (S6) and p70-S6K in ILC tumors. Phosphorylation of 4E-BP1 (Ser65), eEF2, S6 (Ser235/236, Ser240/244), and mTOR (Ser2448) were also significantly lower in LumA ILCs. This lower translation efficiency was then validated in cell lines by O-propargyl-puromycin treatment. Finally, the pathway analysis suggested lower rates of metabolism in lobular tumors. Comparative studies of OXPHOS and glycolysis with a Seahorse analyzer confirmed this finding.

Conclusions

ILC tumors have a higher immune activity than IDC tumors, even with lower rates of stromal inflammation, suggesting a potential for differential response to immunotherapy. The lower rates of translation and metabolism, which are general identifiers of tumor dormancy, could enable ILC to escape from cytotoxic therapies, and may play an important role in the late recurrence of ILC.
Title: Role of PTEN and BRCA1 as determinants of synergy for the combination of vistusertib with carboplatin and olaparib in TNBC

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Body: Introduction: Platinum agents are being used in combination with targeted agents in advanced triple-negative breast cancer (TNBC) (See K. Gelmon et al., 2012). Additionally inhibition of PARP is also being considered as a “targeted” therapy for TNBC (Anders CK et al., 2010). PARP inhibitor (i), Lynparza (olaparib,AstraZeneca)met the primary endpoint of a Phase III trial in which Lynparza was compared to physician's choice of a standard of care chemotherapy in patients with HER2-negative metastatic BC harboring germline BRCA1/2 mutations (BRCAm). Based on cBioportal data analyses and experimental studies we and others have reported that more than 30% PTEN loss in TNBC leads to activation/upregulation of the PI3K pathway (Nature. 2012; Ellis and Perou, 2013; Dey et al., 2012; De et al., 2016; Reed and Shokat 2017;). In line with active kinase profiling, genetic and pharmacological data which defined mTOR as an important target in TNBC (Montero JC et al., 2012), we have demonstrated that mTORi has anti-tumor activity in TNBC (De et al., 2014). Aim: These studies focused on exploring the synergy of biology-based targeted drugs PARPi (olaparib, O), mTOR kinase (vistusertib, V), and platinum (C) in TNBC models. Method: TNBC cells of multiple genetic backgrounds were used to test the combination(s) on proliferation and apoptosis by monitoring growth and using real time Annexin V reagent in a microscopy-based assay (Essen IncuCyte Zoom). Flow cytometric analysis of cell cycle progression by PI staining was also used. Long term clonogenic 3D growth was monitored in matrigel. Results: In BRCAm/PTEN null Sum149 and HCC1937 cells and BRCA wild type (wt)/PTEN null MDA-MB-468 the addition of (V) to (O) and (C) enhanced apoptosis induction and further slowed growth. In Sum149 cells, single agent V treatment induced G1 arrest while O plus C or the triple combination increased S phase accumulation. In MDA-MB-468 cells G1 arrest was seen with V alone and in the triplet. In BRCA wt/PTEN null HCC70 cells V decreased cell proliferation and induced G1 arrest. In the HCC70 model, the addition of O plus C did not synergize with V. In BT20, a BRCA wt/PTEN wt but PI3KCA mutant cell line, no effect on proliferation or apoptosis was seen in the O plus C treated arms. V slowed cell proliferation and increased G1 arrest in a dose-dependent manner. As expected in a BRCA wt/PTEN wt, but RAS active mutant cell line MDA-MB-231 this treatment combination was not effective and was used as an internal negative control. Based on ratios of the normalized slopes of proliferation curves (for triplet), the cells were graded in terms of synergy as SUM149>MDA-MB-468>HCC1937 and HCC70>BT20>MDA-MB231. Treatment with the triplet had the largest effect on reducing 3D colony formation and size as compared to control over single or double treatment. Summary: Here, we present the effect of the combination of vistusertib with olaparib plus carboplatin in several TNBC models. Our data demonstrate that increased effectiveness of the triple combination is seen in cells harboring BRCA1 and PTEN-null mutations. The mechanistic role of these two targets on determining this synergy is being worked out and will be presented at the meeting.
Body: SOX10 is a transcription factor that plays a key role in multiple processes of cellular behaviors in both pathological and physiological processes. In breast cancer biology, the expression of SOX10 is positively correlated with malignancy and prognosis of patients, while the mechanisms under SOX10 mediated malignant phenotypes are not entirely clear. We designed the experimental systems to evaluate SOX10 in promoting progression and metastasis using human triple-negative breast cancer cell line, HCC1806 cells as parental cells. We demonstrated that SOX10 forms a complex with zyxin characterized by co-immunoprecipitation assays and direct binding assays in vitro. Phosphopeptide analyses suggest that $^{45}$S of SOX10 would be modified by phosphorylation. Mutation of $^{45}$S to A in SOX10 ($^{45}$S/A-SOX10) resulted in the failure of the formation of complex with zyxin. In order to characterize SOX10-zyxin complex for regulating gene expressions, we performed microarray studies and demonstrated that the transcription of genes important for breast cancer progression such as TROP2, MSLN, RAB25, CLDN4, CTAG4, MAPK13, PRKCZ, CD74, HPSE, and NEDD9 were significantly upregulated in wt-SOX10 but not $^{45}$S/A-SOX10 transfected HCC1806 cells, suggesting key roles of SOX10-zyxin complex in facilitating cancer metastasis through regulating specific gene expressions. These results were further supported by the results of upregulation of EpCAM, CD44, and ALDH1A1 by wt- SOX10 but not by $^{45}$S/A-SOX10, implicating SOX10 as a regulator of stem cell phenotypes of breast cancer cells. Thus, our results revealed a novel transcriptional regulation by SOX10-zyxin complex and a key residue in SOX10 to mediate the interaction. Given SOX10 is highly expressed in malignant cancer cells, our results suggest that blocking of $^{45}$S of SOX10 is a promising drug target for inhibiting proliferation and metastasis of cancer cells. Furthermore, these results will provide a novel model of the transcription factor-cytoskeletal protein interaction to promote specific gene expressions in microenvironments in the processes of both cancer progression and possibly development.

The views expressed in this article are those of the author and do not reflect the official policy of the Department of Army/Navy/Air Force, the Department of Defense, or U.S. Government.
Title: GNB2 suppresses tumor growth and cancer stem cell load of triple negative breast cancer by controlling STAT3 pathway

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Body: Background: Gametogenetin binding protein 2 (GGNBP2) is encoded in human chromosome 17q12-q23, a region known as a breast and ovarian cancer susceptibility locus. GGNBP2 has a single C2H2 zinc finger and a consensus LxxLL nuclear receptor (NR) binding motif. We have reported that GGNBP2 suppresses ERα-positive breast tumorigenesis by acting as a nuclear receptor co-repressor to restrain ERα activity. However, the detailed molecular mechanisms of GGNBP2 and its role in triple negative breast cancer (TNBC) remain largely unclear.

Methods: A human breast cancer tissue array containing 138 human breast tumor tissues were utilized to examine GGNBP2 expression in breast cancer samples by IHC. To address the potential anti-breast tumor activity of GGNBP2 in vitro, we expressed exogenous GGNBP2 in TNBC cells, including MDA-MB-231 and Cal51 cell lines. Cell proliferation and cell cycle were assessed by cell growth curve/EdU assays and flow cytometry after propidium Iodide staining. Apoptosis was determined by flow cytometry after annexin V staining, by caspase 3/7 and caspase 9 activity assays. Cancer stem cell properties were determined by expression of CD44/CD24/ALDH1 markers. The levels of phosphorylated STAT3 and total STAT3 were determined by western blot. Quantitative PCR and Western blot were carried out to evaluate the effects of GGNBP2 overexpression on STAT3 target genes, CCND1, Mcl-1, survivin, bax and bim expression.

Results: GGNBP2 expression is down-regulated in TNBC cells and patient tumors and it is associated with poor patient survival. Overexpression of GGNBP2 significantly induces cell cycle G0/G1 phase arrest and apoptosis in TNBC cell lines. Expression of cancer stem cell markers also decreased in GGNBP2-overexpressed TNBC cells. GGNBP2 reduces the expression levels of CCND1, Mcl-1 and survivin, promotes the expression levels of bax and bim proteins. Importantly, overexpression of GGNBP2 inhibits STAT3 phosphorylation and STAT3 downstream garget gene expression, including CCND1, Mcl-1 and survivin.

Conclusion: GGNBP2 serves as a critical nuclear negative regulator of STAT3-mediated gene expression and tumorigenesis.
Junctional adhesion molecule-A (JAM-A) is a transmembrane protein with important physiological functions in regulating cell-cell adhesion. Pathophysiologically, its high expression in breast tumour tissue has been shown to correlate with that of Human Epidermal Growth Factor Receptor-2 (HER2), whilst JAM-A knockdown in breast cancer cells has been shown to reduce HER2 expression and signaling. Although HER2 has been successfully targeted in the oncology setting for several years, the problem of clinical drug resistance to HER2-targeted therapies (among other factors) has recently put the spotlight on other HER family members and their upstream regulators as potential drug targets. HER3 is the most potent binding partner of HER2 in activating tumor growth signaling, and accumulating evidence suggests that HER3 plays an important role in resistance to anti-HER2 therapies. Furthermore HER3 is frequently overexpressed in HER2-negative breast cancers, and, along with other HER family members, may drive HER2-independent tumorigenic mechanisms. Since JAM-A levels have been reported to regulate HER2 expression (Brennan et al, Oncogene 2013 32(22):2799-804), we hypothesised that JAM-A also regulates expression of HER3 in breast cancer cells. Results from our study showed that stable overexpression of JAM-A in MCF7 breast cancer cells (MCF7-JAM) increased both mRNA and protein expression of HER3. Correspondingly, transient gene silencing of JAM-A reduced the mRNA and protein expression of HER3 in MCF7 and MCF7-JAM cells. JAM-A silencing also reduced HER3 expression in HER2-positive BT474 breast cancer cells. As the cell lines tested were all Estrogen Receptor-α (ERα)-positive, we examined whether ERα was required to permit JAM-dependent regulation of HER3. However concomitant silencing of ERα in MCF7 cells did not alter the capacity of JAM-A silencing to reduce HER3 protein levels. In MCF7 and MCF7-JAM cells, JAM-A gene silencing phenocopied that of HER3 gene silencing by reducing protein expression of the HER downstream effectors phospho-AKT and phospho-ERK, in parallel with significant reductions in cell viability (measured by Alamar Blue assay). To begin exploring the mechanism whereby JAM-A regulates HER3 expression, we focused on the HER3 transcription factor FOXA1. JAM-A knockdown reduced expression of FOXA1 in MCF7 and MCF7-JAM cells, and knockdown of FOXA1 was sufficient to reduce HER3 expression in the same cells. Taken together, our data provide novel evidence of a direct relationship between levels of JAM-A, FOXA1 and HER3 in breast cancer cells. The relationship appears to be uni-directional, since silencing of HER3 did not alter JAM-A expression in any cell line tested. In conclusion, we suggest that JAM-A merits investigation as a novel target to inhibit HER3-dependent tumorigenic signaling in breast cancer. Our ongoing investigations will determine the pharmacological value of inhibiting JAM-A signaling in breast cancer models, and its potential significance in the setting of resistance to HER2-targeted therapies.
Title: TMEM126A suppresses TGFβ-driven epithelial-to-mesenchymal transition and metastasis in breast cancer by regulating mitochondrial retrograde signaling

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Body: TMEM126A is a one of mitochondrial transmembrane proteins and its functions and mechanisms involved in breast cancer progression remain unclear. Here, by performing SILAC assays in breast cancer primary and metastasis cell models we found that TMEM126A expression was decreased in breast metastasis cell. We further confirmed its expression in breast cancer cells and tissues. Down-regulation of TMEM126A in breast cancer cell lines significantly elevated cell metastasis in vitro and in vivo and overexpression it decreased the metastatic potential. Mechanistic studies by RNA-Seq indicated that TMEM126A may regulate cell metastasis by MAPK, CAMs, Focal adhesion, actin cytoskeleton, TGFβ. Our further study revealed that loss of TMEM126A can activate p-smad3 and promoted cell epithelial-to-mesenchymal transition and actin cytoskeleton rearrangement. What's more, TMEM126A silence induces an increase in production of ROS, depolarization of MMP and cell metabolic disorders, such as increased ATP productin and decreased lactate secretion. Low TMEM126A expression correlated with tumor progression and poor prognosis in patients. Collectively, our study shows that loss of TMEM126A induces mitochondrial dysfunction and subsequently leads to cell metastasis promotion via activating p-smad3 induced epithelial-to-mesenchymal transition and actin cytoskeleton rearrangement. These findings identified TMEM126A as a novel metastasis suppressor and may providing potential new prognostic indicators for breast patients.
Title: Breast tumour kinase and its role in mTOR signalling

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Body: Background: The development of distal metastases and the acquisition of resistance to chemotherapeutic agents are one of the leading causes of cancer related death in breast cancer patients. There is both a scientific and clinical need to understand the alterations in cellular signalling pathways that could contribute to chemotherapeutic resistance in breast cancer, thereby identifying novel targets for therapy. The intracellular tyrosine kinase Brk/PTK6 enhances coupling of ErbB signalling to PI3K/Akt and we hypothesise that Brk plays a role in chemo-resistance and activation of downstream effectors such as mTOR.

Methods: The protein and activation levels of mTOR pathway components and expression levels of Brk, in both Taxol-sensitive and resistant breast cancer cells, were examined by western blotting and immunofluorescence. Brk transfected cells were treated with BEZ-235 for 72 hours and relative cell numbers determine by MTT assay. Immunoprecipitation studies were carried out on lysates from T47D cells, transfected to express FLAG-tagged Brk, with ANTI-FLAGM2 agarose beads. Sequence alignment was done using the NCBI BLAST Tool.

Results: We found that mTOR signalling was up-regulated in the Taxol-resistant cell line compared to parental Taxol-sensitive cells. This up-regulation was also accompanied by increased Brk levels. Transfection of a Brk-negative breast cancer cell line, MDA-MB-468 with wild-type Brk resulted in increased levels of both mTOR and, to a lesser extent, the downstream signalling component GβL at the protein level compared to cells transfected with vector only. Levels of the mTOR inhibitor DEPTOR were also decreased in response to Brk expression and mTOR co-precipitated in FLAG-Brk pull downs using FLAG M2 beads. The Taxol resistant cells also showed altered responses to the mTOR inhibitor RAD-001/everolimus. Interestingly, sequence alignment revealed that there are common amino acid motifs between Brk and the mTOR regulatory molecule DEPTOR.

Conclusions: These data implicate Brk in up-regulating mTOR expression and indicate that Brk may influence mTOR signalling in the development of Taxol resistance. It is possible that Brk could substitute for DEPTOR in mTOR complexes providing a mechanism for elevated mTOR signalling in many breast cancers.
Title: The ratio of omega-3 to omega-6 PUFAs impact cancer cell phenotype in the tumor microenvironment

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Body: Background: Studies have shown that obesity is associated with a worse breast cancer prognosis. Besides the effect of different stages of diagnosis and co-morbidities, recent data from our published in vitro and retrospective studies suggests that this phenomenon may occur because the obese state promotes a more aggressive cancer phenotype through the cyclooxygenase (COX-2) pathway and its production of prostaglandin E2 (PGE2). The metabolization of omega-3 fatty acids decreases the production of PGE2, and have been shown to have potential benefit to cancer patients by decreasing inflammation-related signaling. Our previous clinical trial showed mixed results in the effect of omega-3 PUFA supplements on PGE2 production in post-menopausal obese women. This led us to the hypothesis that the ratio of omega-3 to omega-6 PUFAs have differential effects on cell types within the tumor microenvironment, impacting cancer cell phenotype.

Approach: In vitro experiments, including wound-healing assays to determine motility, and clonogenic assays to determine overall survival, were performed to determine if exposure to higher ratios of omega-6 to omega-3 fatty acids lead to a more aggressive cancer phenotype. MCF-7 breast cancer cells were treated with the following fatty acid ratios of omega-6 (arachidonic acid (AA)) to omega-3 (eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)): 46:1, 20:1, 10:1, and 1.3:1. The wound-healing assays showed greater motility with higher ratios of omega-6 to omega-3 fatty acids conditions and the clonogenic assays showed greater survival with the higher ratios.

Conclusion: These data indicate that lowering ratios of omega-6 to omega-3 fatty acids may lessen the aggressiveness of breast cancer cells and be beneficial to some patients. Studies are on-going to investigate the impact of PUFA ratios on cancer cell phenotype directly, including proliferation and invasion, as well as the indirect effects from modulation of the other cells within the tumor microenvironment, including the macrophages and adipocytes.
Title: A novel oncolytic herpes simplex virus, GD116, has enhanced antitumour efficacy in human breast cancer

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Body: Background: Oncolytic herpes simplex virus-1 (oHSV-1) vectors are promising new therapeutic agents for cancer. For the neurovirulence of ICP34.5 protein, the encoding gene named γ34.5 is deleted in all oHSV-1 mutants currently in clinical trials. However, the deletion of γ34.5 also promotes the interferon-induced shutoff of viral protein synthesis, and attenuates the virus replication in cancer cells at the same time. Previous studies reported that the carboxyl-terminus of MyD116/GADD34 was homologous to that of γ34.5 gene; hence, it might substitute for γ34.5 gene to enhance the replication and cytotoxicity of the virus.

Objective: To reconstruct a novel oHSV-1 mutant named GD116 by inserting γ34.5-MyD116 chimeric gene into G47Δ genome, and to investigate whether the carboxyl-terminus of murine MyD116 can enhance the antitumour efficacy of GD116 on human breast cancer cell line in vitro and in vivo. Methods: Using G47Δ genome and bacterial artificial chromosome (BAC) as the backbone, the GD116 virus expressing γ34.5-MyD116 was reconstructed via Cre/loxp and FLP/FRT recombinase systems. A GD-empty virus mutant containing only the CMV sequence was also constructed as a control at the same time. Then, the replication and cytotoxicity of these two virus mutants in MDA-MB-231 human breast cancer cell line was evaluated in vitro. Meanwhile, the subcutaneous tumor model was also generated by injecting MDA-MB-231 cells into the right flank of BALB/c mice. Then, the antitumor efficacy of GD116 on breast cancer in vivo was evaluated by intratumoral injection. Results: Compared with GD-empty, GD116 possessed an enhanced replication capability and oncolytic efficacy in MDA-MB-231 cell line. On the fifth day after infection with GD116 at MOIs of 0.1 and 0.3, 35.0% and 50.2% of MDA-MB-231 cells were killed respectively, which were higher than that with GD-empty (24.3% and 37.8%). Additionally, GD116 exhibited enhanced antitumor efficacy on subcutaneous tumor xenografts of human breast cancer cells in vivo compared with GD-empty. On day 21 after treatment, the average tumor volume of mice was 585.5 mm³, 289.0 mm³ and 121.9 mm³ in Control, GD-empty and GD116 group respectively. Conclusion: Our findings indicate that the carboxyl-terminus of the murine MyD116 can substitute for the corresponding domain of the γ34.5 gene in oHSV-1 to promote the replication of the virus in infected cancer cells; and the mutant GD116 armed with γ34.5-MyD116 chimaera has enhanced oncolytic effect on MDA-MB-231 breast cancer cells in vitro and in vivo.
Body: The human body harbors ten times more bacterial cells than human cells – a stunning figure that suggests a likely dynamic between our bodies and the bacteria we carry, both in health and disease. In this study, we characterized and compared the gut, oral, and breast tissue microbiomes from women with invasive breast cancer, women with ductal carcinoma in situ (DCIS), and healthy women. Samples were collected prior to any systemic therapy to avoid therapy-associated effects on the microbiomes studied. Kits containing materials for collecting oral and stool swab samples were distributed to patients for self-collection. DNA was isolated from these samples and bacterial 16S rRNA was PCR amplified and sequenced. Based on the sequencing results, bacterial taxa present in the samples were enumerated. In our analyses, we looked at microbial diversity and differential relative abundance of bacterial taxa across the three cohorts. Oral and gut microbial diversity at various taxa levels were assessed using Shannon and Simpson diversity indices. The oral microbiome did not show any significant difference in microbial diversity across the three cohorts. In the gut microbiome, the invasive cohort showed a significant decrease in microbial diversity when compared to the healthy cohort. Differences in phylogenetic and relative abundance of bacterial taxa across the three cohorts were measured using a T-test analysis with a p value less than 0.05 considered significant. In the oral microbiome, there were no significant differences in the relative abundance of bacteria across the three cohorts. In the gut microbiome, there were significant differences in the relative abundance of bacteria within each cohort on the phylum, family, and genus levels. The genus *Fusicanterbacter* (associated with the *Lachnospiraceae* family and *Firmicutes* phylum) was significantly overabundant in gut microbiomes of healthy women when compared to the gut microbiomes of women with DCIS or invasive breast cancer. Meanwhile, the genus *Bacteroides* (associated with the *Bacteroidaceae* family and *Bacteroidetes* phylum) was significantly overabundant in the gut microbiomes of women with invasive breast cancer when compared to the gut microbiomes of healthy women. Although tissues are often thought of as sterile, there is emerging data indicating that different tissues may harbor their own unique microbiomes. We obtained breast tissue microbiome data from a small subset of our breast cancer and DCIS cohorts, as well as healthy breast tissue from reduction mammaplasty specimens. At the genus level, we observed an enrichment of *Lactococcus, Lactobacillus,* and *Halomonas* in healthy breast tissues compared to breast cancer tissues and an enrichment of *Hyphomicrobiunm* in breast cancer tissues compared to healthy breast tissues. Understanding how gut, oral, and tissue microbiomes relate to breast cancer may open up new opportunities for the development of novel markers for early detection (or markers of susceptibility) as well as new strategies for prevention and/or treatment.
**Title:** CRISPR/Cas9-guided editing of spliceosome factors enhances major histocompatibility complex proteins in triple-negative breast cancer

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**Body:** Triple-negative breast cancer (TNBC) exhibits an extraordinary plasticity allowing adaptation to unfamiliar microenvironments and survival despite hindrances imposed by aggressive therapeutic approaches and the immune system. Alternative mRNA splicing (AS), catalyzed by spliceosome factors (SFs), is a major source of transcriptional diversity and phenotypic plasticity for normal and cancer cells. We ascertained that patients with TNBC present up-regulation of specific subsets of SFs mainly involving the epithelial splicing regulatory proteins 1 and 2 (ESRP1/2) and the polypyrimidine tract binding proteins (PTBP1/2). Methods and Results: Through the integration of in-house and publicly-available gene expression profiles (n=890), we evaluated the correlation between the levels of 290 SFs in patients with TNBC. This analysis identified nine subsets of TNBC based on SFs expression profiles with diverse clinical and pathological characteristics. These findings were further validated using data generated by the TCGA-BRCA (n=1,105) and METABRIC projects (n=2,509). Interestingly, up-regulation of PTBP1 was significantly associated with a shorter relapse-free survival interval for patients with TNBC (n=305; HR=1.58 (1.07 – 2.33); p-value=0.02). To systematically identify PTBP1-regulated AS events, we generated and clonally selected CRISPR/Cas9-guided PTBP1 knock-out (KO) TNBC cell lines. Analysis of our RNA-sequencing data at the gene level revealed a significant enrichment of inflammatory response and antigen presentation pathways. Evaluation of potential upstream transcriptional regulators for the enriched molecular pathways and predicted PTBP1 targets identified SMARCA4, a member of the SWI/SNF chromatin remodeling complex, to be significantly activated after PTBP1KO (q-value<0.01). Integration of ENCODE ChIP-sequencing and JASPAR transcription factor binding profile databases revealed clusters of SMARCA4 binding sites upstream of several members of the major histocompatibility (MHC) class I and MHC class II genes. Mechanistically, we identified that PTBP1 induces SMARCA4 exon 30 retention leading to the full length transcript variant 1, which has lower affinity for HLA gene promoter regions. Significant up-regulation of HLA-A, HLA-B, HLA-DPA1, and HLA-DRA genes in CRISPR-guided PTBP1KO TNBC cells was further demonstrated by either western blot or indirect immunofluorescence. Finally, a negative correlation between PTBP1 and HLA genes expression was also identified in multiple breast cancer gene expression datasets. Conclusions: This study suggests that alterations in the PTBP1-associated splicing programming lead to a reduction of the antigen presentation capability of TNBC cells. Due to the limited therapeutic alternatives for patients with TNBC, beyond chemotherapy, further understanding and modulation of this novel alteration may expand the applications of immunotherapy for patients with TNBC.
Title: JNK signaling regulates tumor cell–tumor-associated macrophage cross-talk in triple-negative breast cancer

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Body: Despite advances in our understanding of the molecular mechanisms underlying the aggressiveness of triple-negative breast cancer (TNBC), the contribution of tumor-associated macrophages (TAMs) to TNBC pathogenesis has not been therapeutically exploited. TAMs are the most abundant cell types in the tumor microenvironment (TME) and the key contributor to tumor progression and invasion. We have found that c-Jun NH2-terminal kinase (JNK), a member of the MAPK family and a major regulator of inflammation, contributes to TNBC tumorigenesis by promoting the cancer stem-like cell phenotype. However, whether the JNK pathway regulates TAMs and their cross-talk with tumor cells in TNBC remains unknown. Here, we tested the hypothesis that JNK signaling contributes to TNBC aggressiveness by promoting the tumor cell–TAM cross-talk that facilitates TNBC cell invasiveness.

We found that, among 80 patients with primary inflammatory breast cancer (IBC), TNBC tumors (n=18) had 2-fold more TAMs than non-TNBC tumors (n=62, P=0.05) and that high TAM counts were correlated with shorter disease-free survival of patients with IBC (P=0.05). Both JNK1 and c-Jun were highly activated in TAMs, and JNK-IN-8, a pan-inhibitor of JNK, suppressed c-Jun activation. JNK-IN-8 also increased expression of M1 macrophage markers (CD80 and HLA-DR) but reduced expression of TAM markers (CD163 and CD206), suggesting that JNK suppresses M1 macrophage differentiation but promotes TAM differentiation. Co-culture with TAMs significantly enhanced migration and invasion of HCC70 and MDA-MB-468 human and 4T1 murine TNBC cells. Similarly, an enhancement in TNBC cells migration and invasion was observed following culture with TAM-conditioned medium, suggesting that TAMs enhance TNBC cellular activities through paracrine signaling. In addition, inhibition of JNK signaling in TNBC cells or in TAMs by JNK-IN-8 significantly suppressed TAM-promoted enhancement of TNBC cell migration and invasion. These studies strongly suggest that JNK regulates M1/TAM differentiation and TNBC cell–TAM cross-talk. Furthermore, cytokine/chemokine profiling analysis showed that, of the identified molecules, MCP-1 (secreted by TAMs) and VEGF (secreted by TNBC cells) had the highest expression levels and that their expression was dramatically reduced following JNK-IN-8 treatment. Stimulation with recombinant VEGF increased proliferation of MDA-MB-468 cells, and stimulation with recombinant MCP-1 enhanced migration of the cells. These findings suggest that VEGF and MCP-1 are involved in JNK-mediated TNBC cell–TAM cross-talk.

Together, our results suggest that JNK signaling regulates tumor cell–TAM cross-talk through MCP-1– and/or VEGF-mediated paracrine signaling and that JNK is an important therapeutic target in TNBC. Further animal studies using JNK-knockout TNBC cells co-injected with TAMs are needed to confirm our in vitro findings.
Title: Progestin receptor content of breast cancer associated with expression of gene subsets for peptide/protein hormones and cognate receptors in LCM-procured cells that impact clinical outcomes

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Body: Assessing breast cancer prognosis and treatment selection was first enhanced by discovery that estrogen (ER) and progestin receptor (PR) proteins were clinically relevant biomarkers. Later reports suggested expression of certain protein hormones in breast cancer cells appear to be related to clinical behavior. We deciphered clinically relevant relationships between PR content in tissue biopsies and relative expression levels of genes directing synthesis of peptide/protein hormones and their cognate receptors in LCM-procured breast cancer cells. This global approach revealed unique relationships between both PR+ or PR- carcinomas and small subsets of genes for these peptide/protein hormones and cognate receptors as independent predictors of risk of recurrence.

Methods: Expression of genes for 61 peptide/protein hormones and 81 cognate receptor proteins were measured by microarray analyses of LCM-procured carcinoma cells from de-identified primary breast carcinoma biopsies. Using an IRB-approved biorepository and database, previously total RNA was extracted from carcinoma cells to determine expression levels of ~22,000 genes. Univariable and multivariable Cox regressions with interaction of each hormone receptor gene, individually or paired, and use of LASSO were determined with relative gene expression values of each protein ligand and its cognate receptor. PR content and ligand binding affinity of each carcinoma were quantified by FDA approved assays.

Results: Using de-identified clinical outcomes that extended up to 12 years, univariable Cox regression analyses of 142 candidates revealed AVPR1A, AVPR2, CALCR, CRH, LHB, POMC, SCT, SST, SSTR1 and TMSB10 independently predicted PFS or OS. Violin plots identified candidate genes associated with PR content. Multivariable analyses of relative gene expression of 115 hormone-receptor pairs showed IAPP-CALCR, RLN2-RXFP1, GHRH-GHRHR, CGA-TSHR, EDN1-ENDRA and POMC-MC5R exhibited statistically significant interaction for predicting OS among 145 PR+ cancers. Four gene pairs (HCRT-HCRTR2, CRH-CRHR1, HCRT-HCRTR1 and CORT-SSTR4) were associated with OS among 101 PR- lesions. Using LASSO, PR+ lesions expressing either (CGA & SSTR2) or (CGA) predicted OS and PFS, respectively. Similarly, for PR- cancers, gene subsets (GRP, TMSB15, VIP2) or (AGT, GH1, GRP, TMSB15A) predicted OS and PFS, respectively. Three of four signatures were externally validated with SurvExpress.

Conclusion: Different gene expression profiles for protein hormones and cognate receptors were identified in PR+ or PR- carcinomas at time of diagnosis that predict PFS and OS regardless of treatment. Combining PR content with gene expression of LCM-procured cells is likely to provide insight into alternative treatments whereby standard of care performed poorly or to identify genes that correlate with cancer progression regardless of treatment modality. Collectively, results suggest that many primary breast cancers exhibit considerable endocrine autonomy for controlling disease progression, supporting investigation of protein products of gene candidates in isolated populations of breast carcinoma cells to develop novel biomarker assays.
Title: Hyperthermia regulates transporter expression via ROS production and enhances the cytotoxicity of doxorubicin

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Body: Introduction: Hyperthermia (HT) is a non-invasive cancer therapy. Treatment temperature between 41°C to 44°C has no cytotoxic damage in normal cells, however shows cytotoxicity in cancer cells because of the underdeveloped vascular system. HT often used with other cancer therapy such as radiation therapy and chemotherapy. However mechanism of synergistic effect using these therapies remains unclear. Compared to 37°C, 42°C is mild heat stress for cells, thus superoxide anion is released from tissue. Superoxide anion is produced by mitochondrial electron transport chain. Reactive oxygen species (ROS), produced by mild heat stress, can be released from mitochondria. We have previously reported that ATP-binding cassette sub-family G member 2 (ABCG2) expression was suppressed by increasing mitochondrial ROS, and induction of the cancer specific porphyrin accumulation. ABCG2 is a transporter of doxorubicin (DOX), therefore we hypothesized that synergistic effect of HT and chemotherapy would be induced by down-regulation of ABCG2 expression via intracellular ROS increase. In this study, we investigated if cytotoxic effect of breast cancer cell using DOX can be enhance by HT via intracellular ROS increase.

Materials and methods: The murine breast cancer cell line, 4T1E was incubated at 37°C or 42°C for 1h. Intracellular ROS generation after HT treatment was detected by electron spin resonance (ESR). Twenty four hours after HT treatment, cells were incubated in medium containing 0, 0.1 and 1 µM DOX for 24 h. Cell viability was measured using the Cell Counting Kit 8, a water-soluble tetrazolium-8 based colorimetric assay. ABCG2 expression in whole cells was analyzed by Western blotting.

Results and discussion: ESR signal peak with HT treatment became high as compared to without HT treatment, indicating intracellular ROS level was increased by HT treatment. Cell viability and ABCG2 expression were decreased by DOX exposure and by HT treatment. The enhancement of HT treatment effect by DOX is considered to be result of down-regulation of ABCG2 expression by ROS. When cells were exposed to DOX with 5-aminolevulinic acid (ALA), cell viability reduced further. Since it is known that porphyrin is introduced by ALA and is transported by ABCG2, we speculate that ALA worked as a competitive inhibitor of DOX excretion transporter to enhance cell death. ESR signal peak in ALA treatment cells was higher than that in non-ALA treatment cells. Significant increase in cellular damage by HT treatment was shown by adding ALA, but not without ALA. Moreover, cell death induced by HT and ALA treatment was suppressed by adding N-acetylcysteine (NAC), which is an antioxidant. These results suggest that cellular damage of HT treatment is due to ROS production induced by ALA.

Conclusion: HT treatment involved intracellular ROS production and down-regulated the expression of ABCG2 protein. HT treatment also enhanced the cell damage by DOX. Cell death by DOX was enhanced by combination with HT and ALA treatment, possibly via intracellular ROS generation, and was suppressed by adding antioxidant.
Title: Putting multigene signatures to the test: Prognostic assessment in population-based contemporary clinical breast cancer

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Body: Background
Gene expression signatures hold promise for a molecularly driven division of primary breast cancer with clinical implications. A gap still remains in the application/validation of such signatures in actual clinical treatment groups from unselected, population-based, primary breast cancer receiving current standard of care therapy. We analyzed classification proportions and overall survival (OS) of 14 reported gene expression phenotypes (GEPs) and risk predictors (RPs) in seven clinical treatments groups from an 3273-sample breast cancer cohort representative of population-based disease in the South Swedish healthcare region.

Patients and methods
Between 2010-09-01 to 2015-03-31, 5101 (87%) of 5892 patients with invasive primary disease in the healthcare region were included in the SCAN-B study (ClinicalTrials.gov ID: NCT02306096). Inclusion criteria included no generalized/prior contralateral disease and known surgery/treatment status (neo- or adjuvant). 3273 tumors were profiled by RNA sequencing and matched to clinicopathological patient data from the National Breast Cancer Register, with distribution of clinicopathological characteristics reflecting proportions in the catchment region. RNA profiles were classified according to 14 reported gene signatures featuring both GEPs (PAM50, IC10, CIT, TNBCtype) and specific risk predictors (e.g. Oncotype Dx, 70-gene, 76-gene, ROR-variants, genomic grade index). Classifications were investigated for association with patient OS by univariate and multivariate analyses in seven adjuvant clinical treatment groups: TNBC-ACT (adjuvant chemotherapy, n=228), TNBC-untreated (n=83), HER2+/ER- with trastuzumab + ACT treatment (n=101), HER2+/ER+ with trastuzumab + ACT + endocrine treatment (n=210), ER+/HER2- with endocrine treatment (n=1477), ER+/HER2- with endocrine + ACT treatment (n=637), and ER+/HER2- untreated (n=216).

Results
For the majority of signatures, analysis of classification demonstrated prognostic value limited to ER+/HER2- tumors given follow-up time. Several signatures (including Oncotype Dx, 70-gene, ROR-variants) showed strong predictive value in identifying a subset of ER+/HER2- patients receiving a combination of endocrine and ACT therapy with excellent overall survival (>96%), indicating appropriate therapy selection. In addition, for both ER+/HER2- treatment groups signature analysis identified high-risk groups of patients in clear need of additional treatment beyond standard therapeutic regimes, even with less than 5-years of follow-up.

Conclusions
Our results support the prognostic association of gene expression signatures in large unselected population-based primary breast cancer cohorts even with a short follow-up of OS. Importantly, prognostic associations are limited to specific subgroups for different classifiers and in population-based breast cancer some clinically important subgroups constitute a small proportion of cases. In this context, continued population-based inclusion and broad transcriptional profiling of breast cancer patients provides an opportunity for application to broader patient groups (e.g. TNBC and HER2+), and for consensus classification of individual risk assessments that could potentially provide more stable predictions.
Comparative survival analysis of multiparametric tests in the TEAM pathology study: What to do when molecular tests disagree?

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Body: Multiparametric assays for risk are increasingly used in the management of node-negative and node-positive hormone receptor-positive invasive breast cancer. Data from multiple sources suggests different tests may provide different risk estimates at the individual patient level¹. Analysis from the TEAM pathology study (Bayani and Yao et al npjBreast Cancer, 2017) allows direct comparison of prognostic information from gene signatures in a clinical trial cohort of postmenopausal patients. Risk classifications using genes comprising the following multi-parametric tests: OncotypeDx® (Genomic Health Inc.)²,³, Prosigna® (NanoString Technologies, Inc.)⁴-⁶, Mammaprint® (Agendia Inc.)⁷,⁸ were performed. For the OncotypeDX-Like Recurrence Score (RS), RNA abundance was processed to fit the measurement range as described²,³, with classification into high, intermediate or low risk groups based the derived RS and modeled for DRFS. For the Prosigna-Like Risk of Recurrence Score (ROR), samples were processed as previously outlined⁹, then modelled against DRFS. For the MammaPrint-Like Risk Score, samples were processed by published methods⁸ and modelled for DRFS. Comparing OncotypeDx-Like with Prosigna-Like showed that 45% of cases were classified identically by both (3.3% low risk, 20.9% intermediate, 20.7% high). Of 3370 cases, 353 (10.5%) had scores differing by more than 1 classification (i.e. hi/low or low/high). Almost all (343) of these were cases classified high risk by OncotypeDX-Like RS/low risk by Prosigna-Like ROR (Table 1). Univariate Cox regression analysis, using low/low cases as a reference (relative risk of distant metastasis =1.0), suggested that cases called low risk by Prosigna-Like ROR/High risk by OncotypeDX-Like RS did not perform differently from cases called low risk by both tests (Table 2). However, all cases called intermediate by one test and high risk by another appeared to be high risk (Table 2). Comparisons between Prosigna-Like ROR and MammaPrint-Like scores showed similar concordance between low/low and high/high (52.5% of cases with concordant results). In Prosigna-Like ROR intermediate risk cases, MammaPrint-Like results divided cases between low and high risk, as predicted. Comparisons between these tests is challenging, and evidence on their discordance in risk stratification presents further dilemmas. Preliminary analysis of TEAM suggests a complex inter-relationship between test results in the same patient cohorts requiring careful evaluation.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>OncotypeDX-Like RS</th>
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<tbody>
<tr>
<td></td>
<td>Low</td>
</tr>
<tr>
<td>Prosigna-Like ROR</td>
<td></td>
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<tr>
<td>Low</td>
<td>112</td>
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<tr>
<td>Int.</td>
<td>167</td>
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<tr>
<td>High</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>289</td>
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</table>

<table>
<thead>
<tr>
<th>Table 2</th>
<th>OncotypeDX-Like RS</th>
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<tbody>
<tr>
<td></td>
<td>Low</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Prosigna-Like ROR</th>
<th>Low</th>
<th>Ref</th>
<th>1.26 (0.57-2.79)</th>
<th>1.13 (0.49-2.62)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Int.</td>
<td></td>
<td>1.2 (0.47-3.05)</td>
<td>2.22 (1.03-4.78)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td></td>
<td>6.10 (1.58-23.6)</td>
<td>4.15 (1.79-9.59)</td>
</tr>
</tbody>
</table>
Title: Breast cancer-specific survival (BCSS) in SEER patients with 21-gene Recurrence Score® (RS) results <11 classified as prognostic stage IA by new 8th edition AJCC staging manual

Frederick L Baehner1,2, Valentina I Petkov3, Debbie McCullough1 and Steven Shak1. 1Genomic Health, Inc., Redwood City, CA; 2University of California, San Francisco, San Francisco, CA and 3National Cancer Institute, Bethesda, MD.

Body: Introduction: The 8th edition of the AJCC Staging Manual added molecular features for the first time and now includes ER, PR, HER2, and the 21-gene assay for prognostic staging. We obtained real-world evidence of BCSS by age, tumor size, and grade in a population-based SEER analysis of N0, HR+, HER2-negative breast cancer and RS <11 (tumor size up to 5 cm) to obtain more detailed information on clinically “unfavorable” subgroups.

Methods: RS results were provided to SEER registries as mandated by their methods for linkage (Petkov npj Breast Cancer 2016). Patients (pts) with RS <11 and size up to 5 cm were eligible if N0, HR+, HER2-negative, had no prior malignancy, and were diagnosed between Jan 2004 and Dec 2012 (SEER survival updated through 2013). 5-year BCSS was estimated using actuarial methods.

Results: 9,304 pts had RS <11 (19% of those meeting all other eligibility), with median follow-up of 40 months. Median age was 59 y, with 22% <50 y and 16% ≥70 y. Tumor size was >2 cm in 21% of pts. Tumor grade was moderate in 54% and poor in 8% of pts. Overall, 5-year BCSS for pts with RS <11 was 99.6% (95% CI 99.4%, 99.7%), with reported chemotherapy (CT) use in 3.3% of pts. In contrast, BCSS for pts with RS >25 and RS ≥31 was 3.5% and 4.7%, respectively, with frequent CT use. 5-year BCSS for important clinicopathologic subgroups show high 5-year BCSS despite “unfavorable” age, tumor size, and grade (Table).

5-y BCSS for RS <11, by Clinicopathologic Factors

<table>
<thead>
<tr>
<th></th>
<th>RS &lt;11 and Age &lt;50 y</th>
<th>RS &lt;11 and Tumor Size 2.1-5.0 cm</th>
<th>RS &lt;11 and Poor Tumor Grade</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>N (% of N with CT 'Yes')</td>
<td>5-y BCSS (95% CI)</td>
<td>N (% of N with CT 'Yes')</td>
</tr>
<tr>
<td>2009 (6.2%)</td>
<td>100% (100%, 100%)</td>
<td>1976 (5.5%)</td>
<td>686 (5.8%)</td>
</tr>
</tbody>
</table>

Conclusions: In this SEER population-based study, pts with RS <11 had a wide range of clinicopathologic features. 5-year BCSS was high (>99%), regardless of age, tumor size, or grade. These results support the new AJCC staging criteria that classify N0, HR+, HER2-negative pts with RS <11 and tumor size up to 5 cm as having Prognostic Stage IA disease.
Title: Simplified histological grading of breast carcinoma – potential for improved concordance and consistency in breast cancer grading?

John MS Bartlett\textsuperscript{1,2,3}, Jeremy Thomas\textsuperscript{4}, Elizabeth Mallon\textsuperscript{5}, Tammy Piper\textsuperscript{1}, Jane Bayani\textsuperscript{2}, Annette Hasenburg\textsuperscript{6}, Dirk G Kieback\textsuperscript{7}, Christos Markopoulos\textsuperscript{8}, Luc Dirix\textsuperscript{9}, Caroline Seynaeve\textsuperscript{10}, Cornelis JH van de Velde\textsuperscript{11} and Daniel W Rea\textsuperscript{12}. \textsuperscript{1}Edinburgh Cancer Research Centre, Edinburgh, United Kingdom; \textsuperscript{2}Ontario Institute for Cancer Research, Toronto, ON, Canada; \textsuperscript{3}University of Toronto, Toronto, ON, Canada; \textsuperscript{4}Western General Hospital, Edinburgh, United Kingdom; \textsuperscript{5}Western Infirmary, Glasgow, United Kingdom; \textsuperscript{6}University of Mainz, Mainz, Germany; \textsuperscript{7}Helios Medical Center, Schleswig, Germany; \textsuperscript{8}Athens University Medical School, Athens, Greece; \textsuperscript{9}St. Augustinus Hospital, Antwerp, Belgium; \textsuperscript{10}Erasmus MC Cancer Institute, Rotterdam, Netherlands; \textsuperscript{11}Leiden University Medical Center, Leiden, Netherlands and \textsuperscript{12}Cancer Research UK Clinical Trials Unit, Birmingham, United Kingdom.

Body: Histological grade remains an independent predictor of outcome for invasive breast cancer. The internationally accepted standard grading system is the Elston and Ellis grading system based on a local hospital (Nottingham) cohort treated between 1951-1973. Histological grade, with nodal status, tumour size and receptor measurements (ER, PgR, HER2) give important information even in the context of current molecular testing for breast cancer. In 2009 we proposed a simplified approach to the EE system based on evidence from another hospital series (Thomas et al Histopathology 2009 DOI 10.1111/j.1365-2559.2009.03429.x). Here we report a second validation of this approach using a large phase III clinical trial cohort the Tamoxifen Exemestane Adjuvant multicentre Trial.

A single pathologist (EM) regraded over 4200 cases using a single H&E slide from the TEAM pathology study. Individual scores (1-3) were provided for tubule formation, nuclear pleomorphism and mitotic count and summed to provide the EE score (3-9) resulting in a final grade of 1, 2 or 3 for each case. As previously reported the Simplified Binary Scoring system (SBS) reorganizes this data such that each component is given a score of 1 or 2 with a sum ranging from 3-6. In the current analysis we compared the impact of this revised grading system on patient outcome.

Of 4264 centrally regraded tumours in the TEAM pathology cohort, EE scores for tubular formation were 1 in 102 cases (2.4%), 2 in 503 cases (11.8%) and 3 in 3659 (85.8%). For nuclear pleomorphism only 2 cases were EE score 1 (0.05%), 3117 were score 2 (73.1%) and 1146 score 3 (26.9%). For Mitotic count 3423 (80.3%) were scored 1, 707 (16.6%) scored 2 and 134 scored 3 using the EE system. As previously observed, most/all EE categories could be captured using a simple binary system (SBS, see Table 1).

Table 1

<table>
<thead>
<tr>
<th>EE Grade</th>
<th>SBS SCORE</th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>546</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>2397</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>682</td>
<td>17</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>0</td>
<td>618</td>
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</table>

<table>
<thead>
<tr>
<th>GG Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>EE Grade</th>
<th>SBS SCORE</th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>332</td>
<td>78</td>
<td>19.02%</td>
</tr>
<tr>
<td>2</td>
<td>1377</td>
<td>1322</td>
<td>48.98%</td>
</tr>
<tr>
<td>3</td>
<td>57</td>
<td>517</td>
<td>90.07%</td>
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</table>

<table>
<thead>
<tr>
<th>GG Score</th>
</tr>
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<tbody>
<tr>
<td>Low</td>
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</table>


In a comparison between conventional grading and molecular (using a Genomic-Grade signature) we observed the predicted equal split of EE Grade 2 cases into GG high/versus low. For the SBS score the higher scores were enriched for GG High cases. We show a novel grading system can provides a potentially simple and more reproducible approach to immunohistochemical grading. Comparisons with molecular grading approaches may suggest improved concordance between novel grading approaches and molecular systems. Further comparisons with outcome and molecular signatures will be presented.
Clinical practice and mortality in patients with oncotype DX intermediate score: What do we know about the gray zone?

Abiola F Ibraheem¹, David Press¹, Huo Dezheng¹ and Funmi Olopade¹. ¹University of Chicago, Chicago, IL.

**Body:**

**BACKGROUND:** The 21 gene expression signature Oncotype DX (ODX) is a powerful predictor of disease outcome in early stage hormone receptor positive breast cancer. ODX can be used for better risk stratification of patients who may benefit from chemotherapy. ODX testing increases confidence in treatment decisions especially when ODX recurrence score (RS) is low or high. However, for the intermediate RS, decisions on receiving chemotherapy or not is a joint decision made by the physician and patient, in part because results from the TAILORx and RxPONDER randomized clinical trials addressing this very important clinical question has yet to be published.

**OBJECTIVE:** To examine “real world” oncology practice and survival outcome for breast cancer patients with ODX RS score in the intermediate range.

**METHODS:** A retrospective cohort of women diagnosed with early stage, hormone receptor positive breast cancer whose ODX score was in the intermediate range was established using the National Cancer Data Base (NCDB), 2009-2013. We assessed the relationship of overall survival with chemotherapy receipt and RS score using Cox proportional hazards models adjusted for patient characteristics, including age, race/ethnicity, grade, year of diagnosis, Charlson Co-morbidity Index, lymphovascular invasion, nodal involvement, tumor size, and histology. Intermediate RS score was analyzed as both continuous variable and categorical variables (18-20, 21-23, 24-26, and 27-30).

**RESULTS:** Of 24,945 females reported to have intermediate ODX score, 10,179 (41.4%) received chemotherapy. Receipt of chemotherapy depended on ODX score monotonically, ranging from 19.1% in patients with RS = 19 to 76.1% in patients with RS = 30. In total, 426 patients have died from all causes. Overall, chemotherapy receipt was associated with a statistically significant reduction in the risk of death (hazard ratio=0.77; 95% confidence interval [CI]: 0.62-0.96, p=0.02) in multivariable Cox model. RS score was statistically positively associated with increased risk of death, with one unit increment in RS corresponding to 6.2% increased mortality risk (95% CI 3.2%- 9.2%, p<0.0001). Using a categorical scale, we found patients with intermediate ODX scores in RS 27-30 range had 1.64-fold increased risk of death (95% CI: 1.13-2.37) compared to patients with ODX scores in RS 18-20 range.. The association between ODX score and overall survival was more pronounced in women younger than 50 (hazard ratio per 1 unit increment = 1.28, 95% CI: 1.17-1.40) than in women 50 years or older (hazard ratio per 1 unit increment = 1.04, 95% CI: 1.01-1.07).

**CONCLUSION:** Our analysis revealed the ODX score in the intermediate range still has prognostic value for overall survival especially for young-onset breast cancer patients, which is independent of other clinopathologic factors. As we eagerly await results from randomized clinical trials, young women with intermediate RS should be counseled to consider more aggressive treatment including chemotherapy, ovarian function suppression or participation in ongoing clinical trials of CDK inhibitors to improve overall survival outcomes.
2017 San Antonio Breast Cancer Symposium

Publication Number: P1-06-06

Title: No age-related outcome disparities according to 21-gene recurrence score groups in early breast cancer patients treated by adjuvant chemotherapy in the prospective WSG PlanB trial

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Body: Background: Elderly breast cancer (BC) patients (pts) have been reported to have worse BC-related outcome than younger pts, even within clinical trials such as TEAM. Shak et al. recently showed in a large SEER data analysis that in the high 21-gene recurrence score (RS) group, older pts (≥70y) receive less chemotherapy (CT) and have a worse BC-specific mortality than younger pts. Here, we therefore aimed to see whether there are age-related outcome disparities according to RS groups in pts receiving state-of-the-art CT in the prospective WSG PlanB trial.

Material and Methods: PlanB compared 6 cycles of anthracycline-free TC vs. standard anthracycline-taxane based CT (4xECà4xDoc) in patients with high risk pN0 (T2-4, G2-3, <35 years, or high uPA/PAI-1) or pN+ HER2- early BC. 21-gene assay was performed in all HR+ tumors and omission of chemotherapy (CT) recommended in RS≤11 HR+ pN0-1 BC. Final analysis for the CT randomization for RS 12-25 after 60 months median follow-up revealed similar 5-year DFS and OS outcomes for both CT arms (ASCO 2017).

Results: In all pts with luminal cancer and RS results (n=2577), there was an age-related significant difference in RS risk group assignment (p<0.0001): in young pts (<40y), 9.1% had RS≤11, 52.7% RS 12-25, and 38.2% RS>25; in pts 40-69 years, 18.3% had RS≤11, 61% RS 12-25, and 20.7% RS>25; in elderly pts (≥70y), 19.5% had RS≤11, 55.3% RS 12-25, and 25.2% RS>25. Among patients receiving chemotherapy, RS>25 vs. RS≤25 was associated with significantly poorer DFS separately within the elderly subgroup (HR=3.03, 95%-CI [1.15-7.96]) and in those aged 40-69 years (HR=3.14, 95%-CI [2.18-4.52]); there were only nine events among patients <40y. In particular, among pts receiving CT with RS>25, there were no significant differences in DFS between any two of these three age groups.

Conclusion: A substantial percentage of elderly patients (≥ 70y) presents with high-risk luminal disease; these patients are candidates for CT. In PlanB, about 25% of elderly luminal BC patients had high-risk (RS>25) tumors. Nevertheless, after receiving modern adjuvant CT, their DFS was comparable to that of non-elderly pts with high-risk RS tumors. Consequently, older BC pts with high-risk luminal tumors who are fit enough to receive adjuvant CT should be treated according to guidelines in order to overcome age-dependent survival disparities which have been observed in registries for high-RS tumors.
**Title:** Mayo clinic TNBC outcome calculator: A clinical calculator to predict disease relapse and survival in women with triple-negative breast cancer

Mei-Yin C Polley¹, Roberto A Leon-Ferre¹, Heshan Liu¹, Judith Gilbert¹, Victoria Cafourek¹, David W Hillman¹, Vivian Negron¹, Judy C Boughey¹, Minetta C Liu¹, James N Ingle¹, Krishna Kalari¹, Fergus Couch¹, Daniel W Visscher¹ and Matthew P Goetz¹.

¹Mayo Clinic, Rochester, MN.

**Body:** Purpose: Triple negative breast cancer (TNBC) is an aggressive breast cancer subtype with substantial risks of disease recurrence. While cytotoxic chemotherapy is commonly administered and reduces recurrence, disease outcomes vary considerably and few prognostic tools are available for risk stratification for TNBC patients. We constructed and validated clinical calculators for invasive-disease free survival (IDFS) and overall survival (OS) for TNBC and compared their performance against AJCC-based models which include only tumor size and nodal status.

Methods: From a surgical cohort of 9,982 patients who underwent breast cancer surgery at Mayo Clinic between January 1985 and December 2012, 605 centrally reviewed TNBC patients were identified and used to construct Cox models for IDFS and OS. Patients treated with neoadjuvant chemotherapy were excluded. Variables considered included age, menopausal status, tumor size, nodal status, Nottingham grade, type of breast surgery (mastectomy vs. lumpectomy), adjuvant radiation therapy, adjuvant chemotherapy, Ki67, stromal tumor infiltrating lymphocytes (sTILs), and neutrophil-to-lymphocyte ratio (NLR). Missing values were imputed using single imputation with all variables (including outcomes) included in the imputation model. Backward step-down procedure was used for model selections. The final models were internally validated for calibration and discrimination using bootstrapping methods and compared with AJCC-based models.

Results: For both IDFS and OS, higher sTIL's, less extensive nodal involvement, use of adjuvant chemotherapy, and lower NLR were significant predictors of improved clinical outcomes. Premenopausal status and younger age were additionally predictive of improved IDFS and OS, respectively. Models for IDFS and OS have good calibration and are associated with bias-corrected C-indices of 0.68 and 0.71, respectively, as compared with C-indices of 0.59 and 0.62 for AJCC-based models.

Conclusions: Our data indicate that a clinical calculator that includes sTIL's, NLR, menopausal status, age, nodal involvement as well as chemotherapy use can provide significantly greater prediction of clinical risk than tumor size and nodal status alone. These tools may be used to identify TNBC patients at elevated risk of disease relapse and to aid physician's communication with patients regarding their long-term disease outlook and planning treatment strategies. External validation is required to further evaluate broader applicability of this tool, which was developed utilizing a single-institutional experience.
Title: Independent validation of EarlyR gene signature in E2197: A randomized clinical trial comparing doxorubicin plus docetaxel to doxorubicin plus cyclophosphamide as adjuvant chemotherapy in breast cancer

Sunil Badve¹, Victoria Wang², Scooter Willis³, Brian Leyland-Jones³, Yesim Gokmen-Polar¹, Lawernce Shulman⁴, Silvana Martino⁵, Joseph Sparano⁶, Nancy Davidson⁷, Lori Goldstein⁸ and Steven Buechler⁹. ¹Indiana University, Indianapolis, IN; ²ECOG-ACRIN, Boston, MA; ³Avera Health, Sioux Falls; ⁴University of Pennsylvania, Philadelphia, PA; ⁵The Angles Clinic, Los Angeles, CA; ⁶Montefiore Medical Center, Bronx, NY; ⁷Fred Hutchinson Cancer Center, Seattle, WA; ⁸Fox Chase Cancer Center, Philadelphia, PA and ⁹Notre Dame University, South Bend, IN.

Body: Background: EarlyR is a prognostic gene signature score in ER+ breast cancer (BC) computed from the expression values of ESPL1, SPAG5, MKI67, PLK1 and PGR using a nonlinear mathematical formula. EarlyR has been validated in multiple cohorts profiled on Affymetrix and Illumina microarrays and by RNA-seq. This study sought to assess the prognostic features of EarlyR in a cohort of E2197.

Patients and Methods: Illumina DASL assay was used to measure gene expression in FFPE tissue of primary BC from a case-cohort sampling subset of women in E2197 treated with doxorubicin plus docetaxel (AT) or doxorubicin plus cyclophosphamide (AC). ER+ patients received hormone therapy at physician’s discretion. After 79.5 months median follow-up, disease-free survival was 85% in both treatment arms. Among patients centrally reviewed with sufficient RNA material for the DASL assay, 319 with ER+ status and assessed for EarlyR are included in the analytic cohort. EarlyR scores and pre-specified risk strata (≤25=low, 26-75=intermediate, >75=high) were computed, while blinded to clinical data. The analysis endpoint was disease-free survival (DFS), defined as the time from randomization to date of invasive BC recurrence or death from any cause within 8 years. Weighted Cox proportional hazards models were used to associate EarlyR score or risk strata with DFS. Variances of the estimated coefficients were adjusted to account for the case-cohort design.

Results: The distribution of the EarlyR risk groups was 59% low, 11% intermediate and 30% high risk in this ER+ cohort. The continuous EarlyR score was significantly prognostic of DFS up to 8 years after randomization (p = 0.02). Patients with low EarlyR score (≤ 25) had significantly lower risk of BC recurrence within 8 years (p = 0.031, univariate HR=0.562, 95%CI: 0.334-0.948) compared to those with high EarlyR score (> 75). Analysis within the AC arm showed that patients with low EarlyR score had significantly lower risk of 8-year BC recurrence (p = 0.023, univariate HR=0.392, 95%CI: 0.175-0.878) compared to those with high EarlyR score. Within the AT arm there was no significant difference in 8-year DFS prognosis between any of the EarlyR risk groups.

Conclusions: This study confirmed the prognostic significance of EarlyR using FFPE tissue in a cohort of patients treated with AC chemotherapy from E2197. Patients with high EarlyR score who were treated with AC had significantly higher risk of recurrence than low EarlyR score patients treated with AC. On the other hand, prognosis of high EarlyR score AT-treated patients was not significantly lower than the prognosis of low EarlyR score AT-treated patients. Further study in a larger cohort is needed to assess the relative benefits of AC versus AT within the EarlyR high risk group and the EarlyR low risk group.
Title: Correlation of breast cancer index (BCI) results to lymphovascular invasion in early stage HR+ breast cancer

Manasa Vulchi¹, Max Sagalnik², Catherine A Schnabel² and Jame Abraham¹. ¹Cleveland Clinic and ²Biotheranostics, Inc.

Body: Background: Positive lymphovascular invasion (LVI) is a negative prognostic factor for women with early-stage ER+ breast cancer. LVI, along with other clinicopathologic factors such as larger tumor size, higher grade and positive nodal status, increase a patient's risk of late (post-5y) recurrence. Breast Cancer Index (BCI) is a validated gene expression-based assay for patients with early-stage HR+ breast cancer that reports an individualized risk of late distant recurrence based on a combination of the HOXB13/I17BR ratio and the molecular grade index (MGI). The correlation of LVI and individualized risk stratification by genomic analysis is not well characterized. Therefore, this study evaluated risk stratification by BCI based on the presence or absence of LVI.

Methods: A population of 2,613 patients with known LVI status were identified in the Breast Cancer Index Clinical Database for Correlative Studies, an IRB-approved de-identified database which contains clinicopathologic and molecular variables of more than 19,000 clinical cases submitted for BCI testing. LVI was recorded as either positive or negative based on pathology report review. BCI results based on LVI status from LN- (n=2035) and LN+ patients (n=578) were evaluated separately. Chi-squared tests were used to compare BCI results between LVI groups.

Results: In analyses of 2,613 patients with LVI data available (median age 59.1 y; range 28-89y; 74% ≥50y), 18.3% of patients showed evidence of LVI (LVI-pos). In comparison to the LVI-neg tumors submitted for BCI testing, the LVI-pos tumors had a higher proportion of grade 3 tumors (33% vs 16%, p<0.0001), more LVI-pos tumors were 2.0 cm or greater (45% vs 23%, p<0.0001), a higher percentage LVI-pos patients had node-positive disease (51% vs 16%, p<0.0001), and a higher proportion of LVI-pos tumors showed high Ki67 (Ki67 ≥14%; 64% vs 51%, p=0.004). A correlation between LVI positivity and high BCI prognostic risk was observed, with a higher proportion of LVI-pos patients classified as high risk of late distant recurrence in both the LN- (68% vs 49%, p<0.0001) and LN+ subsets (84% vs 70%, p<0.0001) compared to LVI-neg patients. LVI-pos patients had a higher median molecular proliferative status (MGI) compared to LVI-neg patients regardless of nodal status (p<0.0001 for both). In contrast to the categorical LVI prognostic factor, the wide distribution of BCI individual risk scores provides additional resolution that identifies a substantial subset of LVI positive tumors (32%) that that have a low risk of late recurrence by genomic analysis.

Conclusion: While BCI Prognostic stratification correlated with LVI status, BCI identified a subset of patients with LVI positivity as having a low risk of late distant recurrence that otherwise would have an unfavorable prognosis based on LVI and/or LN positivity. These findings help to characterize differential patient stratification based on an individualized assessment of tumor biology versus LVI for patients considering EET.
Title: Correlative analysis of breast cancer index (BCI) restratification of 21-gene recurrence score (RS) in patients with hormone receptor-positive (HR+), node-negative breast cancer

Fengting Yan¹, Aashini K Master², Mason A Israel³, Junmei Liu³, Catherine A Schnabel³, Sara Hurvitz² and Vijayakrishna K Gadi¹. ¹University of Washington/Fred Hutchinson Cancer Research Center; ²University of California Los Angeles and ³Biotheranostics, Inc.

Body: Background: In a cross-stratification analysis performed within the TransATAC cohort, Breast Cancer Index (BCI) and 21-gene Recurrence Score (RS) had a concordance of 58.2% (Sestak et al., Clin Cancer Res, 2016). Restr stratification by BCI of the low and intermediate RS risk groups led to subgroups with significantly different rates (P < 0.001 and P = 0.003, respectively); in contrast, restratified subgroups created by RS of BCI risk groups did not differ significantly. The objective of this study was to analyze the concordance of BCI and RS test results in HR+, node-negative (LN-) patients tested in the real-world setting and to investigate molecular, clinical, and pathologic correlates within discrepant cases.

Methods: This study utilized a subset of cases from the BCI Clinical Database for Correlative Studies, an IRB-approved de-identified database which contains clinicopathologic and molecular variables from clinical cases submitted for BCI testing. Clinicopathologic variables, abstracted from pathology reports, were available for a subset of cases. This analysis evaluated cases from LN- patients with available RS data. Concordance was evaluated between BCI Prognostic risk groups (Low, High) and RS risk groups (Low, Intermediate, High based on TAILOR Rx cutpoints [0-10, 11-25, and 26+]). Fisher's Exact tests were used to compare molecular (HoxB13/IL17BR [H/I] endocrine response biomarker and Molecular Grade Index [MGI] proliferation marker) and clinicopathologic (age, grade, size, HER2, Ki67) data in discrepant risk groups.

Results: There were 456 LN- patients included. Median age was 58.0y (range 27.2-84.0y; 73.9% ≥50y); 33.1%/50.1%/16.8% were grade 1/2/3; and 24.0%/59.5%/15.3% were T1ab/T1c/T2. BCI classified 47.8% (n=218) of patients as Low Risk vs 52.2% (n=238) as High Risk. RS classified 17.1% (n=78), 67.1% (n=306), and 15.8% (n=72) of patients as Low, Intermediate, and High Risk, respectively. BCI restratified RS-Low patients as high risk in 17.1% of cases, restratified RS-Intermediate as Low Risk in 48.4% and High Risk in 51.6%, and restratified RS-High as Low risk in 20.8% of cases. In RS-Low patients, only H/I (P=0.0004) and MGI (P=0.047) were significantly correlated with restratification to BCI-High Risk. In RS-Intermediate patients, H/I (P<0.0001), MGI (P<0.0001), grade (P<0.0001), and Ki67 >20% (P=0.0003) were significantly correlated with restratification by BCI to High or Low Risk. In RS-High patients, H/I (P=0.0008), MGI (P<0.0001), grade (P=0.016) were significantly correlated with restratification to Low Risk.

Conclusion: BCI restratified a substantial proportion of patients in each RS risk group. Based on previous studies demonstrating that BCI has improved prognostic ability for assessment of risk of late distant recurrence (Sgroi et al., Lancet Oncol, 2013), these results highlight the clinical utility of BCI within all RS risk groups. The estrogen signaling pathway biomarker H/I and proliferative biomarkers (MGI, grade, Ki67) were associated with restratification by BCI, while age, HER2 status, and tumor size were not.
**Title:** Prediction of oncotype DX® results based on local gene expression measurements by MammaTyper®

Hans-Anton Lehr¹, Sebastian Aulmann², Mark Laible³, Alfred Etzrodt², Kerstin Hartmann³, Claudia Gürtler³, Ugur Sahin³ and Zsuzsanna Varga⁴. ¹Institute of Pathology, Medizin Campus Bodensee, Friedrichshafen, Germany; ²OptiPath - MVZ für Pathologie, Frankfurt a.M., Germany; ³BioNTech Diagnostics GmbH, Mainz, Germany and ⁴Institute of Surgical Pathology, University Hospital Zurich, Zurich, Switzerland.

**Body:** Background: Oncotype DX® recurrence score (RS) has emerged as a recommended risk classifier for patients with ER+/HER2- early-stage breast cancer. While RS is one of the most rigorously studied risk scores, it is also one of the most expensive tests, thus remaining beyond reach for a many patients.

The necessity for an affordable method for estimating risk of recurrence has motivated investigations on the correlation between RS and traditional parameters such as IHC for ER, PR and Ki67. However, semi-quantitative IHC lacks standardization across different laboratories especially for Ki67.

In this study we therefore investigated whether the standardized assessment of HER2, ER, PR, and Ki67 on mRNA level could better serve for prediction of low risk RS cases.

**Methods:** ERBB2, ESR1, PGR and MKI67 mRNA expression was measured by RT-qPCR in extracts from FFPE breast cancer samples using the MammaTyper® test. Complete data for RS, IHC, grading and mRNA measurement was available for 198 samples. Tumor subtypes according to St Gallen surrogate definition from 2013 were assigned based on binary mRNA marker classification (pos/neg) according to pre-defined cut-offs. Subtype results were compared to RS risk classes based on commercial and TAILORx-trial cut-offs. RS low risk classification (RS ≤25) based on four IHC markers and grading was estimated using the online tool breastrecurrenceestimator.onc.jhmi.edu and compared to observed RS classes.

Finally, the prediction of continuous RS values by mRNA or semi-quantitative IHC measurement was compared by linear regression and subsequent ROC analysis of prediction models.

**Results:** The distribution of RS risk classes in the set of samples with full data was 21% RS 0-10, 39% RS 11-17, 27% RS 18-25, 7% RS 26-30 and 7% RS >30. MammaTyper® called 38% (76) of the samples as Luminal A-like. From these samples 70% and 99% had RS values below 18 and 25 respectively. Only 1 MammaTyper® Luminal A-like sample had an RS >30.

Estimation of RS according to the online tool resulted in classification of 61% (121) of the samples as low risk (RS ≤25). Of these 74% and 98% of samples had observed RS values below 18 and between 18 and 25 respectively. 2 and 1 samples called as low risk by the online tool had an RS of 26-30 and >30 and, respectively.

In linear regression analysis of IHC against RS only PR and Ki67 were significant predictors (p-values <0.0001 and 0.0128) while when using mRNA values ESR1, PGR and MKI67 were found as predictors of RS in the multivariate model (all p-values <0.0001). On a training set (67% of samples) the IHC based prediction model was correlated to the observed RS with an R² of 0.305 whereas the mRNA based model achieved an R² of 0.489. When the models were applied to training and validation dataset (33% of samples) for prediction of an RS >25 result, the IHC based model had AUCs of 0.887 and 0.836, respectively, while the mRNA based model achieved AUCs of 0.909 and 0.899, respectively.

**Conclusion:** mRNA based prediction of RS was considerably better than prediction based on IHC. As Ki67 IHC standardization is reaching its limits, local gene expression measurements with their high degree of standardization could serve as a safer way for prediction of Oncotype low risk results.
**2017 San Antonio Breast Cancer Symposium**

**Publication Number:** P1-06-12

**Title:** Optimizing the use of oncotype Dx in early breast cancer

Rossana Ruiz¹², Fernando Namuche¹, Claudio Flores¹, Alfredo Aguilar¹ and Henry L Gomez¹. 'Oncosalud, Lima, Peru and ²Instituto Nacional de Enfermedades Neoplasicas, Lima, Peru.

**Body:**

**Background:** Oncotype Dx (ODX) prognosticates the risk of recurrence and predicts the benefit of adjuvant chemotherapy in estrogen-receptor-positive breast cancer (BC). However, its cost makes it prohibitive for many health care systems. Our objective was to develop a model that uses routine clinical and pathological parameters to identify ODX high risk patients which require adjuvant chemotherapy.

**Methods:** We retrospectively reviewed ODX and pathology reports from 190 early BC patients treated between 2014 and 2016 in a specialized cancer center. Our population was divided into a training (n:133) and validation set (n:57). In the training set, among available clinico-pathological variables (age, T, ER, PR, Ki67, Elston-Ellis grade) a multiple linear regression model was carried out to select those significantly associated with ODX. Coefficients of statistically significant variables were used to build an equation. The equation was applied in the training set. These results were confronted to ODX categories. The best threshold for selecting high risk patients was identified in the training set and tested in the validation set.

**Results:** Among the tested variables, tumor size (pT), progesterone receptor (PR), Ki67 and Ellston-Ellis grade were significantly associated with ODX RS (Table 1). The linear predictor is: 

\[(0.2544 \times pT) – (0.0739 \times PR) + (0.0861 \times Ki67) + (5.4232 \times Elston\ grade)\]

The threshold score for this equation was set on 14 to discriminate high from low-intermediate risk patients. The test was able to correctly classify high risk patients with a sensitivity of 78%, a specificity of 72% and a negative predictive value of 98%.

**Conclusion:** With further refinement ODX could be omitted in patients classified as high risk by our predictor therefore restricting and optimizing the use of ODX to a smaller population of patients. The observed ODX distribution in our patients is similar to previously reported series suggesting that this equation could be informative in similar clinical settings. Additional external testing using new datasets is ongoing.
Publish Year: 2017

2017 San Antonio Breast Cancer Symposium

Publication Number: P1-07-01

Title: HLA class I expression is associated with tumor-infiltrating lymphocytes and response and survival after neoadjuvant chemotherapy in hormone receptor-positive, HER2-negative breast cancer

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Body: Background: Interactions between cancer cells and the host immune system influence tumor biology, response to therapy and patient survival and their modulation offers promising new approaches for cancer therapy. The downregulation or loss of HLA class I expression in breast cancer cells might be an effective mechanism to evade the recognition by the immune system facilitating malignant behavior.

Aim: To evaluate the association of tumor-infiltrating lymphocytes (TILs) with HLA class I expression and its theranostic value for therapy response and survival after neoadjuvant chemotherapy.

Methods: HLA class I expression was evaluated by immunohistochemistry in a cohort of 732 pre-therapeutic core biopsies from breast cancer patients treated within the neoadjuvant GeparTrio trial. Patients received anthracycline- and taxane-based neoadjuvant therapy and adjuvant endocrine treatment if hormone receptor-positive (HR+). A publicly available microarray dataset of pre-therapeutic core biopsies from 508 breast cancer patients that received neoadjuvant chemotherapy and endocrine treatment if HR+ was used for validation of the results. The association of HLA class I expression with predefined genomic signatures for immune cell populations was evaluated in publicly available data from the cancer genome atlas.

Results: HLA class I expression was associated with TILs (p < 0.001) and was predictive of better response to neoadjuvant chemotherapy in the subgroup of patients with HR+/HER2- breast cancer (14 % in tumors with high HLA vs. 7 % in tumors with low HLA, p = 0.029). Interestingly, high HLA was also predictive for shorter progression-free survival in univariate analysis (HR 1.590, 95 % CI 1.062—2.380; p = 0.024) and after adjustment to clinical and pathological parameters (HR 1.701, 95 % CI 1.105—2.618; p = 0.016). The results could be validated in the independent microarray-based dataset (HR 1.521, 95% CI 1.088 – 2.129; p = 0.0142). HLA class I was not associated with therapy response or survival in hormone receptor-negative breast cancer. HLA class I was associated with a predefined signature for T-cells and cytotoxic T- cells in the cancer genome atlas dataset (rho = 0.546).

Conclusion: HLA class I expression is associated with better response but shorter progression-free survival in HR+/HER2- breast cancer following neoadjuvant chemotherapy. The underlying mechanisms warrant further investigation.
Title: Evaluation of the prognostic value of CD3, CD8 and FOXP3 mRNA expression in early breast cancer patients treated with anthracycline-based adjuvant chemotherapy

Marinos Tsiatas¹, Konstantine T Kalogeras¹, Kyriaki Manousou¹, Ralph M Wirtz², Helen Gogas¹, Elke Veltrup², Flora Zagouri¹, George Lazaridis¹, Angelos Koutras¹, Christos Christodoulou¹, George Pentheroudakis¹, Constantina Petraki¹, Dimitrios Bafaloukos¹, Dimitrios Pectasides¹, Paris Kosmidis¹, Epaminontas Samantas¹, Charisios Karanikiotis¹, Pavlos Papakostas¹, Meletios-Athanassios Dimopoulos¹ and George Fountzilas¹. ¹Hellenic Cooperative Oncology Group (HeCOG), Athens, Greece and ²STRATYFIER Molecular Pathology GmbH, Cologne, Germany.

Body: Background: Tumor-infiltrating lymphocytes (TILs) have been shown to be of prognostic value in several cancer types. In early breast cancer, TILs have a prognostic utility, as well, especially in HER2-positive and triple-negative breast cancer (TNBC). TILs presence is broadly associated with improved survival, however there is controversy regarding TILs subpopulations. In general, T cell infiltration is higher in non-luminal and more aggressive tumors, like the basal-like subtype. Among TILs subpopulations, CD8-positive T cell infiltration is associated with better outcome, whereas high numbers of FOXP3-positive T regulatory cells are associated with worse outcome in ER-positive tumors and better outcome in HER2-positive and TNBC tumors.

Patients and Methods: Early breast cancer patients, treated with anthracycline-based chemotherapy within two randomized trials (HE10/97 and HE10/00) were included in the study. We evaluated, by qRT-PCR, 826 macrodissected formalin-fixed paraffin-embedded tumor tissue samples for mRNA expression of CD3, CD8 and FOXP3 for potential prognostic significance in terms of disease-free survival (DFS) and overall survival (OS). TILs were evaluated in whole sections as percent of total cells.

Results: Median age was 52.7 years, while 54.2% of the patients were postmenopausal and 79.0% ER/PgR-positive. After a median follow-up of 133.0 months, 255 patients (30.9%) had died and 314 (38.0%) had disease progression. All three mRNA markers were positively correlated with TILs (Spearman's r=0.52 for CD3, 0.41 for CD8 and 0.47 for FOXP3, all p-values <0.001), while Ki67 protein expression was greater in tumors with high mRNA expression (median cut-off) of the markers (Mann-Whitney, all p-values <0.001). Additionally, tumors of higher histological grade and negative ER/PgR status were more frequent in patients with high CD3, CD8 or FOXP3 mRNA expression, as compared to patients with low expression, (chi-square, p-values <0.010). In the univariate analysis, high CD3 and CD8 mRNA expression was found to be of favorable prognostic value for DFS (HR=0.74, 95% CI 0.59-0.92, Wald's p=0.007 and HR=0.76, 95% CI 0.61-0.95, p=0.016, respectively). In multivariate analyses, the association of high CD8 mRNA expression with increased DFS was retained (HR=0.77, 95% CI 0.60-0.99, p=0.048), whereas that of high CD3 mRNA expression was of marginal statistical significance (HR=0.77, 95% CI 0.59-1.01, p=0.059). Moreover, a significant interaction was observed between HER2 status and CD3 mRNA expression with respect to DFS (interaction p=0.032). In the HER2-positive subgroup, the hazard ratio associated with high CD3 mRNA expression was of greater magnitude (HR=0.48, 95% CI 0.30-0.76, p=0.002) compared to the hazard ratio presented above, for the entire cohort. No significant findings were observed for FOXP3 in terms of DFS, while none of the studied markers were of prognostic value for OS.

Conclusions: High CD3 and CD8 mRNA expression in early breast cancer patients is of prognostic value for decreased risk for relapse and, in the future, could potentially be of importance in deciding the most appropriate therapeutic strategy in light of the recent immune-related treatment developments.
Background: Obesity and inactivity are associated with an increased risk of cancer-related and overall mortality in women with early-stage breast cancer, but there are few data in advanced breast cancer.

Methods: C40502 was a Phase III trial of first-line chemotherapy for patients with metastatic breast cancer (MBC). Participants were randomized to weekly paclitaxel, nab-paclitaxel or ixabepilone. Height and weight at the time of study enrollment were abstracted from medical records. After study activation, the protocol was amended to collect physical activity (PA) data. Participants completed the Nurses’ Health Study Exercise Questionnaire, indicating the frequency, type and duration of recreational PA in which they engaged at study enrollment. Metabolic equivalent (MET)-hours of weekly PA (MET-hrs/wk) were calculated using the Ainsworth Compendium. PA was dichotomized to 0-9 or ≥ 9 MET-hrs/wk based on data in early stage breast cancer suggesting that women who engaged in > 9 MET-hrs/wk had lower cancer-specific mortality. Association with clinical endpoints was evaluated using multivariate Cox proportional hazard models adjusting for treatment assignment, age, tumor hormone-receptor status, prior taxane use, disease-free interval and visceral metastases.

Results: 799 patients enrolled in C40502 between 2008 and 2011. Baseline body mass index (BMI) was available for 792 patients and PA data for 500 participants. Median follow up was 60 months. Median age was 56.7 years; 72% of patients had hormone receptor (HR)-positive cancers. Median BMI was 28.6 kg/m² (IQR: 24.7-33.1 kg/m²). Patients engaged in a median of 3.3 MET-hrs/wk (about 1 hour of moderate-intensity PA/wk) (IQR: 0.7-12.7 MET-hrs/wk). Neither BMI nor PA was significantly associated with progression-free (PFS) or overall survival (OS).

<table>
<thead>
<tr>
<th>BMI (kg/m²)</th>
<th>N (%)</th>
<th>PFS (months)</th>
<th>Adj HR</th>
<th>P value</th>
<th>OS (months)</th>
<th>Adj HR</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.5-24.9</td>
<td>209 (26.4)</td>
<td>10.0 (9.1-11.2)</td>
<td>ref</td>
<td>0.48</td>
<td>26.1 (23.3-33.2)</td>
<td>ref</td>
<td>0.54</td>
</tr>
<tr>
<td>25-29.9</td>
<td>248 (31.3)</td>
<td>9.0 (7.6-10.3)</td>
<td>1.00 (0.83-1.22)</td>
<td>22.0 (20.0-25.4)</td>
<td>1.05 (0.85-1.30)</td>
<td></td>
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<tr>
<td>≥30</td>
<td>335 (42.3)</td>
<td>8.7 (7.7-9.7)</td>
<td>0.97 (0.81-1.17)</td>
<td>25.5 (23.1-29.5)</td>
<td>0.95 (0.78-1.16)</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>PA (MET-hrs/wk)</th>
<th>N (%)</th>
<th>PFS (months)</th>
<th>Adj HR</th>
<th>P value</th>
<th>OS (months)</th>
<th>Adj HR</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-9</td>
<td>344 (68.8)</td>
<td>7.9 (7.4-9.2)</td>
<td>ref</td>
<td>0.13</td>
<td>23.6 (20.1-26.8)</td>
<td>ref</td>
<td>0.21</td>
</tr>
<tr>
<td>&gt;9</td>
<td>156 (31.2)</td>
<td>9.8 (8.9-12.0)</td>
<td>0.86 (0.71-1.05)</td>
<td>27.4 (22.3-35.6)</td>
<td>0.87 (0.70-1.08)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There was a trend toward longer PFS and OS in patients who reported PA > 9 MET-hrs/wk vs 0-9 MET-hrs/wk, especially in individuals with HR+ cancers (median PFS 11.7 vs 9.2 months [adj HR = 0.84 (0.66-1.05)] and OS 34.0 vs 26.5 months [adj HR = 0.83 (0.66-1.05)] with PA >9 vs 0- 9 MET-hrs/wk).
Conclusions: In some of the first data looking at the relationship between lifestyle factors and outcomes in MBC, there was no relationship between BMI and PFS or OS in patients receiving first-line chemotherapy for advanced disease. A trend toward improved PFS and OS was seen in multivariate analysis in patients who reported higher levels of PA, but results were not statistically significant and could have been influenced by other patient factors. More information is needed regarding the relationship between PA and cancer outcomes, especially in patients with HR+ cancers.
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**Publication Number:** P1-07-05

**Title:** AIB1 is a new putative prognostic biomarker in the luminal A and B-like (HER2-negative) classification of invasive lobular carcinoma

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**Body:**

**Background:** Estrogen receptor (ER) positive HER2-negative breast cancer comprises 75–80% of all breast cancer. This fraction is even higher (>90%) in invasive lobular carcinoma (ILC). According to the St Gallen surrogate definitions of the intrinsic subtypes, Ki67 and progesterone receptor (PgR) are used to classify these tumors as luminal A- and luminal B-like (HER2-negative). These guidelines are based on information derived from patient materials with mixed histological types, where the vast majority of the patients have invasive ductal carcinoma. The ‘luminal-like classification’ together with histological grade, tumor size and lymph node status is widely used in the clinic for prognostication. The aim of the present study was to investigate if the same markers are applicable for ILC, and furthermore, if additional biomarkers involved in the endocrine signaling system, e.g. Amplified in breast cancer 1 (AIB1) and the putative G protein-coupled estrogen receptor (GPER), might provide complementary prognostic information.

**Patients:** Two hundred and thirty-three (N = 233) well-characterized patients with primary ILC, diagnosed between 1980 and 1991 were included. Forty-two percent of the patients received adjuvant endocrine treatment and 2% received adjuvant chemotherapy. All biomarkers were analyzed immunohistochemically on tissue microarray, whereas histological grade was evaluated on whole sections according to Elston and Ellis (NHG). The primary endpoint was breast cancer mortality (BCM).

**Results:** In univariable analyses with 10-year follow-up, Ki67 (high vs. low), NHG (3 vs. 1+2) and AIB1 (high vs. low) were significantly associated to BCM (Hazard Ratio: 4.7, 95% CI: 2.1–10.4, p <0.001; HR: 3.1, 95% CI: 1.5–6.4, p = 0.003; HR: 3.2, 95% CI: 1.4–7.2, p = 0.005 respectively), whereas PgR (<1% vs ≥1%) and GPER (linear 0-4) were not (p = 0.25; p = 0.31 respectively). Essentially the same effect was seen after multivariable adjustment for lymph node status (+ vs. -), tumor size (>20 mm vs. ≤20 mm), adjuvant treatment and age (continuous). Subgrouping the tumors into luminal A- and B-like (HER2-negative) according to St Gallen surrogate definitions did not show significant prognostic differences between the two groups (p = 0.12). Patients with ≤20 mm, lymph node negative breast cancer and favorable tumor characteristics (low Ki67, NHG 1+2, and low AIB1) had a 10-year BCM of 4.2% (95% CI: 1.4–12%). This group constituted 34% of the patients included in the present study.

**Conclusions:** In contrast to other previous studies, where breast cancers of mixed histological types were included, PgR was not significantly associated to prognosis in the ER-positive HER2-negative subgroup in the present study, consisting only of ILC. The prognostic role of PgR and the clinical usefulness of the luminal A and B-like (HER2-negative) classification (using only Ki67 and PgR) in ILC is still to be further investigated. The prognostic importance of Ki67 and NHG in this subgroup was, however, confirmed also in ILC, and AIB1 might be a new putative prognostic factor. By combining Ki67, NHG, and AIB1, together with lymph node status and tumor size, a group of patients with an excellent prognosis could be identified.
Title: The relationship between Klintrup-Makinen score and cancer-specific survival in primary operable breast cancer

Elizabeth S Morrow¹, Fadia Gujam¹, Zahra MA Mohammed¹, Donald C McMillan¹ and Joanne Edwards². ¹Academic Unit of Surgery, University of Glasgow, United Kingdom and ²Institute of Cancer Sciences, University of Glasgow, United Kingdom.

Body: Introduction
It is increasingly being recognised that cancer prognosis is dependent on a complex interaction of tumour factors and the host response. The degree of inflammatory response at the invasive tumour edge, as measured by the Klintrup-Makinen score, has been shown to have prognostic relevance in some cancers but its role in breast cancer remains unclear.

Aim
To evaluate the relationship between Klintrup-Makinen score and prognosis in primary operable breast cancer.

Methods
Patients who underwent surgery for primary operable invasive breast cancer between 1995 and 2007 were studied. Full section haematoxylin and eosin slides from surplus tissue from each breast cancer were analysed. Each was visually scored for the level of inflammatory infiltrate at the invasive edge of the tumour, according to Klintrup-Makinen criteria. Kaplan Meier survival analysis was performed using SPSS.

Results
1195 patients were included in the study, of which 298 had a Klintrup-Makinen score (KM) of 0 (no inflammatory cells at the invasive edge), 589 had a score of 1, 238 had a score of 2 and 70 had a Klintrup-Makinen score of 3 (high inflammatory cell infiltrate). 833 (69.7%) patients were ER positive and 172 (14.4%) patients were HER2 positive. Median follow up was 158 months (28-183) and there were 234 cancer deaths. Patients with the highest and lowest KM scores had the best prognosis (10 year breast cancer specific survival (BCSS) 84% for KM score 3 and 82% for KM score 0), while those with KM score 2 had the worst prognosis with 67% 10 year cancer specific survival (p=0.003). When analysed by subtype, in ER negative patients 10 year BCSS was 95% in KM 0 patients, 80% for KM 3, 72% for KM 1 and 67% for KM 2 (p=0.082). Conversely, in HER2 positive patients, the best prognosis was seen in patients with KM 3 with 86% 10 year BCSS but patients with KM 0 had the worst prognosis (BCSS 62%), but this did not reach significance (p=0.544).

Conclusion
The Klintrup-Makinen score appears to have a prognostic role in primary operable invasive breast cancer, however there is a suggestion that it varies between tumour subtypes. Further work is required to further define this role for each molecular subtype.
Purpose: Incidence of LRs in patients (pts) treated for HR+ HER2- localized BC and distribution overtime have not been described in recent years after introduction of new generation of adjuvant therapies and more extensive use of radiotherapy. We evaluated the incidence and distribution overtime of LRs in pts with HR+ HER2- N+ BCs who entered PACS 01 and PACS04 trials.

Patients and Methods: Data were analyzed from 2909 pts with HR+/HER2- BC out of 5008 included in both trials. Pts underwent mastectomy or lumpectomy plus axillary dissection for a localized N+ BC and, according to study design, were randomized to: 6 cycles of FE100C (standard arm) versus FE100C x 3 cycles followed by docetaxel 100 mg/m2 x 3 cycles (FEC-D) (PACS01) or 6 cycles of Epirubicin 75mg/m2 and Docetaxel 75 mg/m2 (ED75)(PACS04). Loco-regional radiotherapy was mandatory after lumpectomy and recommended in other cases. All pts received 5 years of hormone therapy (HT). A competing risk multivariate analysis was conduct using Fine and Gray model to identify risk factors associated to isolated LRs. Competing events were nodal recurrence, contralateral BC, distant metastasis and death. Cumulative incidence associated to each event was estimated by a Kaplan-Meier estimator.

Results: Pts’ median age was 50 (22-65); 67.2% underwent lumpectomy, 32.8% mastectomy; 67.6% had 1 to 3 N+, 32.4% more than 3 N+; 45.7% had lymphovascular invasion; 49.5% received FE100C, 35.8% ET75, 14.7% had FEC-D; while radiotherapy was given to 97.3% and HT to 92.2%, of whom 90.5% received tamoxifen. At a median follow-up of 9.1 years, 60 pts (2.1%) experienced LR as first event. The 5-year and 10-year cumulative incidence of LRs were 1.04% and 2.53%, respectively. The cumulative incidence of LRs increased from the 5th year, and the annual risk tended to remain constant over time. Multivariate analysis of competing risk showed that younger age, conservative surgery and omission of HT (not prescribed or non-adherence) were independently associated with risk of developing LRs.

Table 1. Multivariate analysis on competing risk of predictors of LRs

<table>
<thead>
<tr>
<th>Variables</th>
<th>HR 95%CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at entry (&lt;35 years, ≥ 35)*</td>
<td>0.95 [0.92; 0.99]</td>
<td>0.009</td>
</tr>
<tr>
<td>Mastectomy, lumpectomy</td>
<td>0.39 [0.17; 0.86]</td>
<td>0.020</td>
</tr>
<tr>
<td>&gt; 20mm, ≤20 mm</td>
<td>0.68 [0.37; 1.24]</td>
<td>0.203</td>
</tr>
<tr>
<td>N+ &gt;3, 1-3</td>
<td>1.73 [0.99; 3.02]</td>
<td>0.055</td>
</tr>
<tr>
<td>Grade II/III, I</td>
<td>1.06 [0.50; 2.24]</td>
<td>0.885</td>
</tr>
<tr>
<td>PR+,PR-</td>
<td>1.78 [0.70; 4.53]</td>
<td>0.223</td>
</tr>
<tr>
<td>Type of chemotherapy 3FEC-3D, 6FEC/6ET</td>
<td>1.32 [0.65; 2.69]</td>
<td>0.446</td>
</tr>
<tr>
<td>Number of cycles 6, &lt;6</td>
<td>0.71 [0.17; 0.75]</td>
<td>0.630</td>
</tr>
</tbody>
</table>
Conclusion: Our analysis showed that incidence of LRs in pts with HR+ N+ BCs treated within PACS trials were considerably lower as compared to earlier studies. These findings may reflect differences in treatment era, as the more extensive use of radiotherapy and new generation of adjuvant chemotherapy. Despite current adjuvant strategies, young age at diagnosis and omission of HT remain independent risk factors of LRs.
Title: Young age and the risk of disease recurrence as assessed by the 70-gene signature – an analysis from the EORTC 10041/BIG 03-04 MINDACT trial

Kim Aalders¹, Els Genbrugge¹, Coralie Poncet¹, Anne Kuijer², Barbara Pistilli³, Martine Piccart⁴, Konstantinos Tryfonidis¹, Thijs van Dalen², Fatima Cardoso⁵, Laura van ‘t Veer⁶ and Emiel Rutgers⁷. ¹European Organisation for Research and Treatment of Cancer (EORTC) Headquarters; ²Diakonessenhuis Utrecht; ³Institut Gustave Roussy; ⁴Institut Jules Bordet, Universite Libre de Bruxelles; ⁵Champalimaud Cancer Center; ⁶UCSF Helen Diller Family Comprehensive Cancer Center and ⁷Netherlands Cancer Institute/Antoni van Leeuwenhoek.

Body: Purpose: Increased insight in tumor biology has revealed that not all young women are at high risk of disease recurrence. Therefore, in some patients extent of treatment could probably be safely scaled down. We aimed to evaluate the risk of breast cancer (BC) relapse according to the 70-gene signature (70-GS) result in relation to young age, in early-stage BC patients enrolled in the MINDACT trial.

Patients and Methods: The analyzed population consisted of enrolled BC patients in the MINDACT trial with available clinical (C), as per a modified version of Adjuvant!Online, and genomic (G), according to the 70-GS, risk assessments and known age (n=6693). Patients were categorized in three age groups; <45 (young), 45-55 (peri-menopausal) and >55 years (post-menopausal). Clinicopathological and treatment characteristics as well as gene expression were compared for the different age groups further split by corrected risk groups (C-low/G-low, C-low/G-high, C-high/G-low, C-high/G-high). Subsequently, the 5-year distant metastasis-free survival according to risk category was calculated.

Results: The study included 1100 patients <45 (16%), 2272 aged 45-55 (34%) and 3321 patients >55 years of age (50%). Median age of the young group was 41 (25.8-45.0) years. The young age group had a higher frequency of lymph node involvement (25% vs. 22% and 19%), poorly differentiated tumors (42% vs. 26% and 27%), ER-negative tumors (20% vs. 11% and 11%) and triple negative molecular IHC subtype (16% vs. 9% ad 8%). Median tumor size was the same across the 3 age groups (17mm). Of the 1100 young patients, 61% were C-high while the 70-GS assessed 48% as G-high. Overall, 31% were CL/GL (vs. 43% in other age groups), 9% CL/GH, 21% CH/GL and 40% CH/GH (vs. 24% and 25%).

In the discordant risk groups, chemotherapy (CT) allocation when randomized to no chemo occurred in 5% of young women as compared to 3% and 1% in the older age groups. Reason for non-compliance was 50/50 between patient refusal and PI decision. Overall, the 5-year DMFS was 94.1% (95% CI 92.4-95.4) in <45 age group, 95.3% (95% CI 94.2-96.1) in 45-55 and 94.9% (95% CI 94.0-95.6) in >55. For the young patients, 5-year DMFS was 98.3% for the CL/GL (96.0-99.3), 97.4% in CL/GH (90.0-99.4), 95.5% in CH/GL (91.6-97.7) and 89.2% in CH/GH (85.6-92.0). In the older two age groups (45-55 and >55), the 5-year DMFS rates were 97.8% (96.5-98.6) and 97.2% (96.2-98.0) for CL/GL, 93.9% (88.8-96.7) and 94.5% (91.0-96.7) for CL/GH, 94.5% (92.0-96.3) and 95.4% (93.5-96.8) for CH/GL and 92.0% (89.2-94.1) and 90.4% (88.0-92.4) for CH/GH, respectively. With 9 events in the <45 group at a CH/GL risk, numbers were too small to evaluate chemotherapy effect in this population.

Conclusion: The use of the 70-GS reduces the proportion of patients characterized as high risk as compared to traditional clinical risk assessment (48% vs. 61%). Outcome was comparable for the 3 age categories with a very good 5-year DMFS of 95-98% in all GL groups. Performing the 70-GS provides clinically relevant information concerning the prognosis for young early-stage BC patients categorized as CH. These results add important new data to the limited available evidence on genomic expression in young BC patients.
2017 San Antonio Breast Cancer Symposium

Publication Number: P1-07-09

Title: Serum activin A and outcomes in HR+/HER2- metastatic breast cancer patients treated with everolimus: Results from BOLERO-2

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Body: Background: Everolimus (EVE) plus exemestane (EXE) doubled progression-free survival (PFS) while maintaining quality of life versus EXE alone in postmenopausal women with hormone receptor positive (HR+), HER2-negative metastatic breast cancer (mBC) (BOLERO-2 phase 3; NCT00863655). Pretreatment serum activin A was previously reported as a prognostic factor in first-line hormone therapy (letrozole vs tamoxifen) (Novartis P025) and anti-HER2 mBC (lapatinib vs trastuzmab) (CCTG MA.31) trials. Here we investigate the prognostic and predictive ability of activin A in BOLERO-2.

Methods: Activin A levels were determined on pretreatment serum samples using ELISA. Cox-proportional hazards model was used to assess the efficacy of EVE in the activin A low and high subgroups (median cut-point), and the prognostic effect of activin A on PFS and overall survival (OS).

Results: Baseline activin A levels were determined in 513 patients (71% of 725 BOLERO-2 patients randomized 2:1 to EVE+EXE or EXE). Predictive and prognostic signals are shown in the table below.

<table>
<thead>
<tr>
<th>Predictive/Prognostic</th>
<th>End-point</th>
<th>Act-inin A</th>
<th>Treatment</th>
<th>N</th>
<th>Events</th>
<th>Median PFS</th>
<th>HR (95% CI); p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>predictive</td>
<td>PFS</td>
<td>H</td>
<td>EXE</td>
<td>93</td>
<td>83</td>
<td>2.5 (1.5-2.8)</td>
<td>-</td>
</tr>
<tr>
<td>predictive</td>
<td>PFS</td>
<td>H</td>
<td>EVE+EXE</td>
<td>163</td>
<td>132</td>
<td>5.4 (4.1-6.8)</td>
<td>0.46 (0.34 - 0.60); &lt;0.0001</td>
</tr>
<tr>
<td>predictive</td>
<td>PFS</td>
<td>L</td>
<td>EXE</td>
<td>89</td>
<td>77</td>
<td>4.2 (2.0 -5.4)</td>
<td>-</td>
</tr>
<tr>
<td>predictive</td>
<td>PFS</td>
<td>L</td>
<td>EVE+EXE</td>
<td>168</td>
<td>105</td>
<td>9.9 (8.1-12.5)</td>
<td>0.38 (0.28 - 0.51); &lt;0.0001</td>
</tr>
<tr>
<td>predictive</td>
<td>OS</td>
<td>H</td>
<td>EXE</td>
<td>93</td>
<td>68</td>
<td>20.1 (13.8-22.6)</td>
<td>-</td>
</tr>
<tr>
<td>predictive</td>
<td>OS</td>
<td>H</td>
<td>EVE+EXE</td>
<td>163</td>
<td>129</td>
<td>17.7 (15.7-22.3)</td>
<td>1.04 (0.78 - 1.40); 0.78</td>
</tr>
<tr>
<td>predictive</td>
<td>OS</td>
<td>L</td>
<td>EXE</td>
<td>89</td>
<td>39</td>
<td>NA (34.7-NA)</td>
<td>-</td>
</tr>
<tr>
<td>predictive</td>
<td>OS</td>
<td>L</td>
<td>EVE+EXE</td>
<td>168</td>
<td>72</td>
<td>41.4 (36.4-NA)</td>
<td>1.02 (0.69 - 1.50); 0.93</td>
</tr>
<tr>
<td>prognostic</td>
<td>PFS</td>
<td>H</td>
<td></td>
<td>256</td>
<td>215</td>
<td>4.1 (2.9-4.2)</td>
<td>-</td>
</tr>
<tr>
<td>prognostic</td>
<td>PFS</td>
<td>L</td>
<td></td>
<td>257</td>
<td>182</td>
<td>6.9 (6.7-8.5)</td>
<td>0.54 (0.45 - 0.66); &lt;0.0001</td>
</tr>
<tr>
<td>prognostic</td>
<td>OS</td>
<td>H</td>
<td></td>
<td>256</td>
<td>197</td>
<td>18.0 (16.5-21.1)</td>
<td>-</td>
</tr>
<tr>
<td>prognostic</td>
<td>OS</td>
<td>L</td>
<td></td>
<td>257</td>
<td>111</td>
<td>42.3 (38.5-NA)</td>
<td>0.34 (0.27 - 0.42); &lt;0.0001</td>
</tr>
</tbody>
</table>

In multivariate analysis (including sensitivity to prior hormone therapy and visceral disease), activin A remained a significant independent prognostic factor for PFS and OS [HR 0.57 (0.46-0.69) and 0.34 (0.27-0.43), respectively].

Conclusions: Higher serum activin A was strongly associated with shorter PFS and OS in HR+/HER2- mBC patients. Everolimus was efficacious regardless of serum activin A level. These results are similar to our previous studies in phase 3 trials of letrozole-tamoxifen (Novartis P025), and HER2-targeted therapy, lapatinib vs trastuzmab (CCTG MA.31): pretreatment serum activin A was prognostic for outcome, but was not a predictive factor for treatment arm selection.
Title: Molecular subtypes of triple negative breast cancer have different sensitivity signature to established chemotherapy agents

Balázs Győrffy1, Ádám Nagy1, Libero Santarpia2 and Christos Hatzis3. 1MTA TTK, Budapest, Hungary; 2Humanitas Clinical and Research Institute, Milan, Italy and 3Yale Comprehensive Cancer Center, New Haven, CT.

Body: Background: Triple negative breast cancer (TNBC, ER/PR/HER2 negative) patients have the worst prognosis among all breast cancer patients. Recently, six molecular subtypes have been proposed to sub-divide TNBC into clinically relevant sub-cohorts. It is unknown whether these molecular subtypes have different sensitivity against known chemotherapy agents. Here, we identified the cross-section of predictive and prognostic genes in a large TNBC dataset.

Methods: An integrated database comprising 2,071 TNBC patients with transcriptome-level gene expression data and clinical follow-up was set up from 52 different GEO and EGA datasets. The database included n=418 basal-like 1, n=170 basal-like 2, n=465 immunomodulatory, n=384 mesenchymal, n=207 mesenchymal stem-like, and n=427 luminal androgen receptor samples. The prognosis was computed for all genes (n=54,675) in each of the six subtypes separately using Cox proportional hazards regression. Another database (n=1,641) was set up for tumors with known therapy response as defined by pathological complete response (PCR) – in this, the correlation to response against a taxanes/anthracycline chemotherapy was computed using Mann-Whitney analysis. The overlap between the two datasets was determined at p<0.0001.

Results: The proportion of responder patients was lowest in basal like 2 subtype. There were more genes significant for response to anthracyclines (n=6,118) than to taxanes (n=3,927). When comparing each subtype separately, most genes were prognostic in basal-like 1 and luminal androgen receptor subtypes (n=73/110 and n=73/111 respectively for taxane/anthracycline response genes). Almost no genes were overlapping to the immunomodulatory and mesenchymal stem-like subtypes (n=8/14 and n=3/7, respectively). When comparing anthracycline and taxane treatment across all six subtypes, there was no significant difference. We have to note that the different sample sizes in the different TNBC cohorts provide different power to detect an association.

Discussion: Based on our results, TNBC tumors in the basal-like 1 and luminal androgen receptors are most likely to benefit from chemotherapy while there is no difference for the selection between the two most common agents.
2017 San Antonio Breast Cancer Symposium

Title: Risk factors for the development of brain metastases (BM) in 506 patients with HER2-positive breast cancer (HER2+ BC): A single institutional retrospective analysis

Christian Maurer¹,², Lorraine Tulip¹, Cristina Dumitrescu¹,², Evandro de Azambuja¹, Michel Moreau⁴, Marianne Paesmans⁴, Jean-Marie Nogaret⁵, Martine Piccart¹ and Ahmad Awada¹. ¹Medical Oncology Clinic, Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium; ²University of Cologne, Cologne, Germany; ³Medical Oncology Clinic, Charleroi University Hospital, Université Libre de Bruxelles, Charleroi, Belgium; ⁴Unité de Gestion de l'Information, Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium and ⁵Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium.

Body: Introduction: BC is the most common malignancy in women. HER2+ BC is the 2nd leading cause of BM after lung cancer. The prognosis of BC patients with BM is poor. Determining risk factors for the development of BM is warranted in order to establish more accurate screening for patients at risk.

Material and Methods: This is a retrospective analysis of patients with HER2+ BC treated at Institut Jules Bordet, Belgium, between 2000 and 2014. Patients’ charts were reviewed for disease characteristics, treatment regimens for primary and metastatic disease as well as clinical outcomes. Statistical analyses were conducted with SAS 9.4 using Cox regression analyses, log-rank test and Kaplan-Meier method.

Results: A total of 506 patients with HER2+ BC were included in the analysis. Median age was 52.7 years (range, 21.5-89.2 years). In total, 138 (27.3%) were diagnosed with metastatic BC and 74 (14.6%) had BM. Among the 138 metastatic patients, 12 (8.7%) had BM as a first site of metastatic disease and 3 (2.2%) developed BM as only site of distant relapse. Median overall survival (OS) for patients with BM was 1.74 years (range, one month-3.2 years). In multivariate analysis, risk factors for the development of BM at time-point of initial BC diagnosis were de-novo metastatic disease (hazard ratio [HR] 4.46; p<0.0001), postmenopausal status (HR 16.65; p<0.0001), and adjuvant breast radiotherapy (HR 1.79; p=0.0198). Identified risk-factors for BM at the time-point of metastatic disease were postmenopausal status (HR 1.90; p=0.0464), the presence of lung metastases (HR 2.61; p=0.0004), an interval of more than one year between initial BC diagnosis and development of metastatic disease (HR 1.91; p=0.0179) and age of <40 years (HR 1.78; p=0.0353). In this cohort, biological factors such as hormone receptor status, degree of HER2 amplification and tumor grade had no impact on the development of BM both in univariate and multivariate analysis. Furthermore, the type of systemic treatment in the adjuvant or metastatic setting (chemotherapy, anti-HER2 treatment) did not modulate the risk for BM. The combination of local treatment (surgery, stereotactic radiosurgery [SRS] or whole brain radiotherapy [WBRT]) and anti-HER2 directed therapy as first-line therapy for BM was associated with an improvement in overall survival (OS) that was more pronounced for tyrosine kinase inhibitors (HR 0.13; 95% confidence interval [CI] 0.05-0.33); p<0.0001) than for trastuzumab and/or pertuzumab (HR 0.53; 95% CI 0.24-1.15; p=0.1085).

Conclusion: The development of BM is a common complication in patients experiencing metastatic HER2+ BC. Until now, no imaging screening for BM is recommended in this patient population. Compared to WBRT, surgery and SRS are associated with an improvement in OS and/or less cognitive impairment. However, these treatment approaches are limited to patients with less extensive BM disease. In this context, randomized trials examining the role of MRI screening for BM in metastatic HER2+ BC with high risk features are warranted.
**Title:** Correlation of breast cancer index (BCI) prognostic and predictive results to clinicopathologic risk groups in early stage HR+ breast cancer

Jose Mayordomo¹, Carla Falkson², Jeffrey Kepes³, Mason A Israel³, Brock E Schroeder³, Catherine A Schnabel³ and Anthony Elias¹. ¹University of Colorado; ²University of Alabama and ³Biotheranostics, Inc..

**Body:**

**Background:** Both clinicopathologic factors and genomic tests have been shown to be prognostic for risk of late distant recurrence (DR); however, few studies have characterized differential patient stratification. Breast Cancer Index (BCI) is a validated gene expression assay for patients with early-stage HR+ breast cancer that provides a prognostic result for high vs low risk of late distant recurrence and a separate predictive result (based on the HoxB13/IL17BR [H/I] ratio) for high vs low likelihood of benefit from extended endocrine therapy. Thus four categories of results are possible based on a patient's tumor biology. To better understand how patient stratification is affected by a combination of clinicopathologic and genomic factors, this study examined BCI assay results within clinicopathologic risk categories based on tumor size and grade.

**Methods:** This study utilized data from the BCI Clinical Database for Correlative Studies, an IRB-approved de-identified database which contains clinicopathologic and molecular variables from 19,126 clinical cases submitted for BCI testing. Clinicopathologic variables, abstracted from pathology reports, were available for a subset of these cases. This analysis evaluated cases from LN- patients with available clinicopathologic data. Chi-squared tests were used to compare BCI results between tumor size and grade subgroups.

**Results:** Analyses included 3843 LN- patients (median age 59.1y; range 26-89y; 74% ≥50y), of which 31%, 52%, 17% were Grade 1, 2, and 3, respectively, and 5%, 22.7%, 48.9%, 21.7%, and 1.6% were T1mi/a, T1b, T1c, T2, and T3, respectively. In analysis based on tumor size, there was a wide distribution of individual BCI Prognostic scores in all tumor size subsets; however, the proportion of patients classified as high risk increased with larger tumor size (T1a/b 39.0%, T1c 50.1%, T2 61.0%; p<.0001). In contrast, BCI Predictive (H/I) was not as strongly correlated with size, with a modestly larger proportion of patients classified as High H/I with larger tumor size (T1a/b 37.2%, T1c 40.5%, and T2 45.3%; p=.005). Within each tumor size category, the proportion of patients classified as BCI High Risk and High H/I increased with tumor grade (p<.0001). However, there was a wide distribution of individual risk assessments by BCI Prognostic and stratification by BCI Predictive (H/I) in all size + grade subsets. In patients with the most favorable clinicopathologic risk profile (T1a/b, G1), BCI classified 20% as high risk, 68% of whom also had High H/I.

**Conclusion:** While BCI results correlated with tumor size and grade, BCI identified substantial proportions of patients with favorable clinicopathologic features as high risk for late DR and apparent high likelihood of benefit from EET; conversely, BCI also identified patients with high risk clinicopathologic features as low risk for late distant recurrence and apparent low likelihood of benefit from EET. These findings help to differentiate between genomic-based and clinicopathologic-based risk/benefit assessment for patients considering EET.
Obesity is associated with poor prognosis of Japanese breast cancer, especially in ER positive/HER2 negative subtype, which tendency is prominent

Masako Sato¹, Sayuri Terai¹, Hanae Tachikawa¹, Hideki Maeda¹, Mitugu Yamamoto¹, Nobumoto Tomioka¹, Kenichi Watanabe¹ and Masato Takahashi¹. ¹NHO Hokkaido Cancer Center, Sapporo, Hokkaido, Japan.

Body: Introduction: Body mass index (BMI) is defined as a poor prognostic factor in breast cancer patients in western countries. Since the percentage of the overweight differs between Asian and western breast cancer patients, we do not know if breast cancer prognosis in Asia is related to obesity, the same as in western countries. Therefore, we have investigated the association between BMI and the prognosis of Japanese breast cancer patients.

Patients and Methods: This study is a retrospective analysis of the 1,924 primary Japanese female breast cancer patients with clinical stage I through III disease to have undergone surgery between January 2004 and December 2013 at the Hokkaido Cancer Center. The data of BMI were at the time of diagnosis, and stratified into 2 groups as non-Obese (BMI < 25 kg/m²), Obese (BMI ≥ 25 kg/m²). The overall survival (OS) and disease-free survival (DFS) were compared between two BMI groups using the Kaplan-Meier method and Cox hazards model.

Results: The number of non-Obese group was 1,353 (70.3%) and Obese group was 571 (29.7%) of the 1,924 patients. Six hundred and thirty two patients were in premenopausal (32.8%), 1,289 were in postmenopausal (67.0%) and 3 were unknown. The median follow-up period was 73 months. Breast cancer recurred in 239 patients (12.2%), and 204 patients died. There were 110 cases of breast cancer-related death, 48 cases of non-breast cancer death, and 46 cases of unknown as the cause of death. Patients in Obese group had shown significantly poorer OS (adjusted hazard ratio (HR) = 1.68, 95% confidence interval (CI) = 0.45 to 0.79) and DFS (HR = 1.46, 95% CI = 1.16 to 1.82). As investigating by subtype analysis, a high BMI in ER positive/HER2 negative patients was associated with a significantly worse OS (HR = 2.04, 95% CI = 1.42 to 2.92) and significantly worse DFS (HR = 1.61, 95% CI = 1.23 to 2.11). On the other hand, there was no significant interaction found between the BMI and OS or DFS in ER negative/HER2 negative patients and HER2 positive patients. Subsequently, when analysis was limited to ER positive HER2 negative, there was a difference in the degree of association between obesity and prognosis due to the difference in menopause status. In premenopausal patients with ER positive/HER2 negative cancer, Obese group had significantly poorer OS (HR = 2.83, 95%CI = 1.32 to 5.88) and significantly poorer DFS (HR = 2.41, 95%CI = 1.41 to 3.99). In postmenopausal patients, Obese group patients had significantly poorer OS (HR = 1.71, 95%CI = 1.11 to 2.58) and poorer DFS (HR = 1.35, 95%CI = 0.98 to 1.86), however. The relevance was not so large compared with that in premenopausal patients.

Conclusion: Among Japanese breast cancer patients, Obese (BMI ≥ 25 kg/m²) was one of poor prognostic factors. Specifically, obesity was associated as an extremely strong prognostic factor in ER positive/HER2 negative premenopausal patients. Since hormone therapy has been practiced in almost all ER positive breast cancers, the relationship between hormonal therapy susceptibility and obesity is noticed not only in western patients but also in Asian patients.
Title: Real-life analysis evaluating >1000 N0/N1mi estrogen receptor (ER)+ breast cancer patients for whom treatment decisions incorporated the 21-gene recurrence score (RS) result: Clinical outcomes with median follow up of > 9 years

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1Davidoff Center, Rabin Medical Center, Petah Tikva, Israel; 2Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; 3Lin Medical Center, Haifa, Israel; 4Soroka University Medical Center and the Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel; 5Oncotest Division, Teva Pharmaceutical Industries, Ltd, Shoham, Israel; 6BioInsight Ltd, Zichron Yaakov, Israel; 7Genomic Health Inc., Redwood City; 8Meir Medical Center, Kfar Saba, Israel; 9Tel-Aviv Sourasky Medical Center, Tel Aviv, Israel; 10Sharet Institute of Oncology, Hadassah-Hebrew University Medical Center, Jerusalem, Israel; 11Rambam Health Care Campus, Haifa, Israel; 12Oncology Institute, Shaare Zedek Medical Center, Jerusalem, Israel; 13Community Division, Clalit Health Services, Tel Aviv, Israel and 14Kaplan Medical Center, Rehovot, Israel.

Body: Background: The 21-gene Recurrence Score (RS) Assay (Oncotype DX®) is a validated prognosticator and predictive of chemotherapy (CT) benefit in patients with hormone receptor (HR)+ human epidermal growth factor receptor 2 (HER2)-negative breast cancer. In Israel, the RS assay has been reimbursed by Clalit Health Services (CHS, the largest HMO in Israel) since 2006, and the assay is widely used in eligible estrogen receptor (ER)+ patients. Notably, ER+ breast cancer patients have a protracted risk of recurrence with approximately half of all distant recurrences occurring after 5 years from diagnosis. The goal of the current ongoing analysis was to investigate early (≤ 5 years) and late (>5 years) distant recurrence in N0/N1mi ER+ HER2-negative breast cancer patients who were RS-tested through CHS.

Methods: This analysis of the CHS registry included breast cancer patients with ER+ HER2-negative N0/N1mi disease who underwent RS testing from 1/2006 (CHS approval of the assay) through 1/2009. Data sources included CHS claims arms (for patient/tumor characteristics), Teva Pharmaceuticals (for tumor characteristics, RS result), and medical records (for treatment/recurrence/survival). The study was approved by the institutional review boards of the CHS Community Division and was granted a waiver for obtaining patient consent.

Results: The analysis included 1026 patients with median (interquartile range) follow up of 9.3 (8.8-10.2) years. Most patients were females (99%). Median (range) age was 59 (25-84) years; 92% had N0 and 8% had N1mi disease; 14%, 52%, and 16% had grade 1, 2, and 3 tumors, respectively (grade information was not available for 18% of patients); median (range) tumor size was 1.5 (0.3-6.5) cm. The majority of patients (78%) had invasive ductal carcinoma and 12% had invasive lobular carcinoma. Overall, 489 patients (48%) had RS<18, 434 (42%) had RS 18-30, and 103 (10%) had RS≥31. The use of adjuvant CT was consistent with the RS result: 3%, 27%, and 90% of RS<18, RS 18-30, and RS≥31 patients, respectively. Overall, 25 distant recurrences were reported within 5 years of RS testing: 5 (1.0%) in RS<18 patients, 9 (2.1%) in RS 18-30 patients, and 11 (10.6%) in RS≥31 patients. In the first 5 years, breast cancer-specific death was reported in 8 patients including 3 (0.7%) with RS 18-30 and 5 (4.9%) with RS≥31 results. Among N0 patients with RS 11-25 who did not receive adjuvant CT (n = 540), 5 (0.9%) distant recurrences and one (0.2%) breast cancer death were reported within 5 years of RS testing. Analysis of ‘late’ recurrences and breast cancer-specific death (from 5 to 9.3 years of follow-up) is ongoing.

Conclusions: These will be the first late recurrence data from over 1000 patients for whom the RS result was used in real-life clinical decision making. Consistent with previous analyses of the CHS registry, CT use was appropriately based on the RS result, and the recurrence/survival outcomes (for the first 5 years) demonstrated the prognostic performance of the RS. Distant recurrence and breast cancer death beyond 5 years will be presented at the meeting.
Title: Retrospective analysis of oncotype DX recurrence score (RS) and discordance in patients with node-negative, ER+ breast cancer with recurrence

Brittney S Zimmerman¹, Krystal P Cascetta¹, Meng Ru¹, Lauren Eggert³, Madeline C Molot², Anupma Nayak⁴, Ira Bleiweiss⁴ and Amy Tiersten¹. ¹Mount Sinai Hospital and Icahn School of Medicine, New York, NY; ²Barnard College of Columbia University, New York, NY; ³Stanford University Hospital, Stanford, CA and ⁴Hospital of the University of Pennsylvania, Philadelphia, PA.

Body: BACKGROUND: Oncotype RS is a 21-gene assay used to predict the likelihood of distant recurrence and benefit of chemotherapy in patients with node-negative, tamoxifen treated breast cancer. We developed a database to determine tumor recurrence rates and identify cases of discordance between Oncotype RS and tumor grade (TG). Our goal was to recognize patients with discordant tumors who had breast cancer recurrence and to understand the implications for patient management.

METHODS/RESULTS: We analyzed patient and tumor characteristics from 704 breast cancer patients between 2006-2016. Of these patients, there were thirteen recurrences (n=13), or 1.9% recurrence rate at a median follow-up of 4.2 years. When stratified by RS, recurrence rates were 1%, 2.4% and 4.3% in low, intermediate and high-risk groups respectively. Of the 13 patients who recurred, 31% had a low RS (<18), 54% had an intermediate RS (18-30) and 15% had a high RS (>31). The median RS was 23 and median age at time of recurrence was 55 years (62% postmenopausal). Tumor characteristics at time of recurrence were notable for: 77% metastatic, 23% locally recurrent, 85% PR positive, 69% moderately-differentiated (MD) and 31% poorly-differentiated (PD). No well-differentiated (WD) tumors recurred.

We defined Oncotype discordance as either 1-step or 2-step difference between Oncotype risk group (low, intermediate, high) and tumor grade (WD, MD, PD). Prior studies have demonstrated 7-19% “2-step discordance” between TG and RS (i.e. PD tumors with low-risk RS or WD tumors with high-risk RS). Of the 13 recurrences in our database, 46% were at least 1-step discordant, compared with 64.3% in our overall database. Among these recurrences, we compared discordant versus concordant tumors using two-sided T-tests. We found that fewer patients were treated with systemic chemotherapy in the discordant group (p=0.045), which was statistically significant. Among discordant patients, only one received chemotherapy, though all displayed MD or PD tumor grade. Discordant tumors tended to have lower RS (mean 17 vs. 27, p=0.34) and tended to be larger (mean 1.88cm vs. 1.33cm), however this was not statistically significant (p=0.84). Notably, the two largest tumors were both discordant. There were no significant differences in terms of age, Oncotype ER/PR score or mitotic count.

CONCLUSION: Although the sample size of recurrent patients is small, our data may suggest that patients with discordant tumors of low-risk Oncotype RS but higher TG may be receiving inadequate treatment (i.e. no chemotherapy). In addition to RS, other factors such as discordance, TG and tumor size should perhaps be considered when determining treatment plans.

Characteristics of Breast Cancer Recurrences in Oncotype DX Database

<table>
<thead>
<tr>
<th>Subject #</th>
<th>Age (years)</th>
<th>Oncotype RS</th>
<th>Tumor Grade</th>
<th>Discordance</th>
<th>Path PR%</th>
<th>Tumor Size (cm)</th>
<th>Chemotherapy</th>
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<tr>
<td>1</td>
<td>41</td>
<td>11</td>
<td>MD</td>
<td>Y</td>
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<td>N</td>
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<tr>
<td>2</td>
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<td>Y</td>
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<tr>
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<td>PD</td>
<td>N</td>
<td>5</td>
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</tr>
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</table>

MD=moderately-differentiated, PD=poorly-differentiated, Y=Yes, N=No, U=Unknown
2017 San Antonio Breast Cancer Symposium

Publication Number: P1-07-17

Title: Discordant breast cancer: Genomic verse clinicopathologic

LeAnn M Blankenship¹, Daniel Ezekwudo¹, Ishmael Jaiyesimi¹, Michael Stender¹, Osama Alassi², Cynthia Kresge¹ and Susanna Gaikazian¹. ¹Beaumont Health, Hematology and Oncology, Royal Oak, MI and ²Beaumont Health, Pathology, Royal Oak, MI.

Body: Background: Studies using the 21-gene recurrence score (RS) have shown early-stage, low-risk pathologic and genomic breast cancers do not benefit from systemic chemotherapy (CTx) whereas early stage, high-risk breast cancers have improved outcomes when treated with CTx. Data is lacking for patients with discordant risk factors and which feature, genomic or clinical, plays more of a role in determining outcomes.

Methods: A retrospective analysis was conducted to identify early-stage breast cancer patients with discordant features, defined as low-risk genomic/high-risk pathologic factors (LG/HP) or high-risk genomic/low-risk pathologic factors (HG/LP), from August 2011–December 2016. LG/HP breast cancer was defined as a RS <18 with ≥2 high-risk pathologic factors: tumor size (T) ≥2cm, lymph node (N) positivity, or grade 2-3 disease. HG/LP breast cancer was defined as a RS ≥31 with all three low-risk pathologic factors: T <2cm, N negativity, and grade 1-2 disease.

Results: There were 469 patients with low-risk RS identified of whom 118 (25%) met discordant high-risk pathologic criteria and 62 patients with high-risk RS of whom 14 (23%) met discordant low-risk pathologic criteria. Thirty patients in the LG/HP group received CTx despite a low RS. Of the 118 LG/HP patients, there were 22 (19%) breast cancer recurrences; 21 with locoregional and one with metastatic disease. Of the locoregional recurrences, 10 were contralateral breast whereas 11 were in-breast recurrence despite breast conservation therapy. Of the 14 HG/LP discordant patients, of whom 12 received CTx, 3 (21%) had breast cancer recurrence; one with metastatic disease to the lung and the other two with contralateral breast cancer. Majority of all recurrences occurred >5 years after initial diagnosis. Staging and management depicted below.

Management (Mgt) of Discordant Risk Cancers

<table>
<thead>
<tr>
<th></th>
<th>LG/HP initial diagnosis (n=118)</th>
<th>LG/HP recurrence (n=22)</th>
<th>HG/HP initial diagnosis (n=14)</th>
<th>HG/HP recurrence (n=3)</th>
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<tr>
<td>Stage</td>
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<td></td>
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<tr>
<td>0</td>
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<td>4</td>
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<td>IA</td>
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<tr>
<td>IB</td>
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<td>IIIA</td>
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<td>0</td>
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<td>0</td>
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<tr>
<td>IV</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
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<tr>
<td>Surgical Mgt</td>
<td></td>
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<tr>
<td>Partial mastectomy</td>
<td>73</td>
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<tr>
<td>Simple mastectomy</td>
<td>43</td>
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<tr>
<td>LN Mgt</td>
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<tr>
<td>Sentinel LN biopsy</td>
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<td>10</td>
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<tr>
<td>Axillary LN dissection</td>
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<td>3</td>
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<td>6</td>
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**Conclusions:** Using traditional low-risk RS of 18, we observed more than expected recurrences in our LG/HP discordant patients. Thus suggesting, in patients with discordant results, clinicians must consider both pathologic and genomic factors to optimize patient-specific treatment. Further studies are needed to improve the outcomes of this unique patient population.

<table>
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<tr>
<td>No/Refused</td>
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<td>19</td>
<td>2</td>
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</table>

*metastatic pt with SBRT to lung*
Title: Clinicopathologic and molecular correlates of breast cancer index (BCI) results in patients with HR+, LN- breast cancer that are high risk of late distant recurrence (DR) / low likelihood of benefit from extended endocrine therapy (EET)

Melanie Royce¹, Graham Poage², Mason A Israel², Catherine A Schnabel² and Frankie A Holmes³. ¹University of New Mexico Comprehensive Cancer Center; ²Biotheranostics, Inc. and ³Texas Oncology.

Body: Background: BCI is a gene expression assay for patients with early stage HR+ breast cancer that provides 2 results: BCI Predictive, based on the HoxB13/IL17BR (H/I) ratio, reports a prediction of high vs low likelihood of benefit from EET; BCI Prognostic, based on the algorithmic combination of H/I and a set of proliferation-based genes, reports the risk of late distant recurrence (DR). Clinical actionability is distinct based on the 4 possible combinations of prognostic and predictive results. To better characterize patients classified by BCI as having a high risk of late DR but a low likelihood of benefit from EET, we assessed clinicopathologic and molecular correlates in this subset.

Methods: The BCI Clinical Database for Correlative Studies is a de-identified database containing >50 clinicopathologic and molecular variables from cases submitted for BCI in clinical practice (N=19,126). Clinicopathologic variables abstracted from pathology reports were available for a subset of these cases. Molecular proliferation status (molecular grade index [MGI]) and clinicopathologic parameters were examined in the 4 possible BCI result categories of BCI Prognostic (High vs Low risk) and BCI Predictive (High vs Low H/I). Chi-squared tests and ANOVA were used to compare BCI results within subsets.

Results: Analyses included 3843 LN- pts with clinicopathologic data: Median age was 59.1y (range 26-89y; 74% ≥50y); 30.9%, 51.7%, and 17.4% were Grade 1, 2, and 3, respectively; 27.8%, 48.9%, 21.7%, and 1.6% were T1a/b, T1c, T2, and T3, respectively. BCI categorized 41.4% of pts as having Low risk/Low likelihood of benefit, 31.3% with High risk/High benefit, 18.0% with High risk/Low benefit, and 9.3% with Low risk/High benefit. Patients with High Risk/Low Benefit had increased median proliferation scores (MGI), and a greater proportion of pts with grade 2/3 tumors and high Ki67 scores compared to pts with Low Risk/Low Benefit (P<.0001 for all). In contrast, there were only modest differences in clinicopathologic parameters between patients with High Risk/ Low Benefit and those with High Risk/High Benefit.

Conclusion: In characterizing the molecular and clinical correlates in BCI cases with a high risk of late DR but low likelihood of benefit from EET, we found that higher proliferative status was associated with classification of high risk of DR. Future studies might investigate whether patients with this molecular pattern might benefit from combinatorial therapy (e.g., CDK 4/6 inhibitors) with EET. This study highlights the importance of predictive biomarkers for individualized EET therapy recommendation.
Title: Protective effect of 27-hydroxycholesterol on disease recurrence in HER2-negative luminal type of early breast cancer

Sim Sung Hoon¹, Park In Hae¹, Jung So-Youn¹, Lee Seeyoun¹, Kong Sun-Young², Kang Han-Sung¹, Lee Eun Sook¹ and Lee Keun Seok¹. ¹Center for Breast Cancer, Research Institute, National Cancer Center, Goyang-si, Gyeonggi-do, Korea and ²Graduate School of Cancer Science and Policy, Center for Diagnostic Oncology, National Cancer Center, Goyang-si, Gyeonggi-do, Korea.

Body: Background: 27-hydroxycholesterol (27-HC) is a metabolite of cholesterol (Chol) and known as endogeneous selective estrogen receptor modulator (SERM). Laboratory researches showed high 27-HC could stimulate cancer cell proliferation and promote metastasis processes in hormone receptor positive breast cancer. However, there is no clinical data about the effect of 27-HC on disease recurrence in early breast cancer.

Methods: A total of 539 early breast cancer patients who underwent surgery at the National Cancer Center Hospital between 2001 and 2010 with the deposition of blood samples at Tumor Bank were accrued. The blood samples were taken with overnight fasting at surgery day. Serum 27-HC was analyzed by liquid chromatography–mass spectrometry (LC-MS). 27-HC and Chol were categorized into two groups (low vs high) by median, respectively.

Results: Hormone receptor was positive in 441 patients and negative in 98 while HER2 was positive in 81, negative in 325 and unknown in 133 patients. The median follow up time was 92 months (range 24-153.7). The 27-HC (median, 115.73 ng/ml with the range of 29.36-234.0) and Chol (median, 170.0 mg/dl with the range of 91-290) showed weak correlation (\( \rho = 0.24, P < 0.001 \)). While 27-HC was not significant in analysis for RFS in all cases, it showed marginal significance for RFS in the subset analysis with HER2-negative luminal breast cancer patients (HR, 0.463; 95% CI, 0.208-1.031; \( P = 0.059 \)). When the patients were grouped according to 27-HC and Chol level (group \( 27\text{HC}/\text{Chol} \)) in luminal subgroup, it showed a trend for increasing RFS in the order of group \( \text{low/high} \), group \( \text{low/low} \), group \( \text{high/low} \) and group \( \text{high/high} \) (\( P_{\text{trend}} = 0.009 \)).

Conclusion: Serum 27-HC was not associated with poor RFS. High serum 27-HC could be related to good prognosis in HER2-negative luminal type of breast cancer, which may be related to the protective effect of 27-HC from disease recurrence. Further study is warranted.
Title: Developing prognostic indicators of poor outcomes in PRAEGNANT metastatic breast cancer cohort

Christopher Szeto\(^1\), Stephen Benz\(^1\), Andy Nguyen\(^1\), Matthias Rübner\(^2\), Diethelm Wallwiener\(^2\), Hans Tesch\(^4\), Peyman Hadji\(^5\), Tanja N Fehm\(^6\), Wolfgang Janni\(^7\), Friedrich Overkamp\(^8\), Diana Lueftner\(^9\), Michael P Lux\(^2\), Markus Wallwiener\(^10\), Matthias W Beckmann\(^2\), Hanna Huebner\(^2\), Johannes Ettl\(^1\), Andreas D Hartkopf\(^6\), Volkmar Mueller\(^12\), Florin Andrei Taran\(^3\), Erik Belleville\(^13\), Andreas Schneeweiss\(^14,10\), Patrick Soon-Shiong\(^15\), Shahrooz Rabizadeh\(^15\) and Peter A Fasching\(^2\).

\(^{1}\)NantOmics, LLC, Santa Cruz, CA; \(^{2}\)Erlangen University Hospital, Comprehensive Cancer Center Erlangen-EMN, Erlangen-Nuremberg, Germany; \(^{3}\)University of Tuebingen, Tuebingen, Germany; \(^{4}\)Oncology Practice at Bethanien Hospital, Frankfurt, Germany; \(^{5}\)Northwest Hospital, Frankfurt, Germany; \(^{6}\)University Hospital Duesseldorf, Düsseldorf, Germany; \(^{7}\)Ulm University Hospital, Ulm, Germany; \(^{8}\)Oncologionova GmbH, Recklinghausen, Germany; \(^{9}\)Charité University Hospital, Berlin, Campus Benjamin Franklin, Berlin, Germany; \(^{10}\)University of Heidelberg, Heidelberg, Germany; \(^{11}\)Klinikum rechts der Isar, Technical University of Munich, Munich, Germany; \(^{12}\)Hamburg-Eppendorf University Medical Center, Hamburg, Germany; \(^{13}\)ClinSol Gmbh & Co KG, Wuerzburg, Germany; \(^{14}\)National Center for Tumor Diseases, Heidelberg University Hospital, Heidelberg, Germany and \(^{15}\)NantWorks, LLC, Culver City, CA.

Body: Background: Despite novel, targeted therapies, metastatic breast cancer patients have an extremely unfavourable prognosis. Prognostic and predictive factors for patients with advanced breast cancer are not well understood. Molecular assessment of the patient and the tumor in the metastatic situation is not routinely performed despite advances in molecular precision medicine indicating great benefit to this patient group. Here we present early findings from the first 142 patients of a prospective molecular breast cancer registry with completed transcriptomic profiling.

Methods: The PRAEGNANT study protocol (NCT02338767) is a molecular registry designed to provide an infrastructure for the real-time comprehensive analysis of tumor and patient molecular characteristics under study conditions. Formalin fixed paraffin embedded tumors have been used from this registry to identify molecular, transcriptomic predictors for overall survival (OS). Known clinical correlates for OS (e.g. hormone-receptor status, age at diagnosis, and BMI) were analyzed by Cox proportional hazard ratios, and compared to transcriptomic markers of outcomes. Transcriptomes for all patient tumors were sequenced on the Illumina sequencing platform, and analyzed by RSEM to estimate transcripts per million (TPM) values for each gene isoform. Log-TPM values were used in established (PAM50) and novel (hierarchical clustering) expression-based subtyping of tumor samples. Expression-based subtypes were demonstrated to be strong prognostic indicators by Cox analysis. A Lasso regression machine learning algorithm was used to develop an expression-based predictive model of OS.

Results: Hormone receptor positivity (HR=0.7, p<0.006) and TNBC status (HR=1.4, p<0.01) were significantly associated with outcomes. PAM50 subtypes were also strong indicators of outcomes (e.g. Basal disease compared to Luminal-A subtype has HR=1.4, p<0.017). A novel expression-based high-risk cluster in this cohort was more indicative of poor prognosis than clinical variates or Basal-type, with a HR=2.7 (p<0.009) when compared to Luminal-A subtype. An expression-based survival prediction model achieved a concordance-index of 0.65 in an unseen validation cohort. Patients predicted as having the shortest survival times were in the high-risk cluster.

Conclusions: Here we demonstrate using molecular profiling to develop prognostic signatures that out-perform standard clinical correlates of poor outcomes, even in a small subset of the total cohort. As the PRAEGNANT cohort expands these prognostic tools will continue to improve and supplement physician knowledge to improve patient outcomes.
2017 San Antonio Breast Cancer Symposium

Publication Number: P1-07-21

Title: Relationship between hereditary cancer syndromes and oncotype DX recurrence score

Sarit Toltzis¹, Nicole Casasanta¹, Shawn Lipinski², Amy Marino³, Allison McHenry¹, Neelima Denduluri², Patricia Rodriguez² and Rebecca Kaltman¹. ¹GW Cancer Center, Washington, DC; ²Virginia Cancer Specialists, US Oncology Network, Arlington, VA and ³University of Pittsburgh Medical Center, Pittsburgh, PA.

Body: Background
Oncotype DX Recurrence Score (RS) is used to stratify breast tumors into those likely to respond to cytotoxic chemotherapy. Women and men with hereditary cancers tend to have tumors that are chemosensitive. We hypothesize that a high RS may harbor a signal of potential hereditary risk. This analysis aims to identify whether breast cancer patients with hereditary cancer syndromes have a disproportionate amount of high RS compared to sporadic cases.

Methods
Individuals with a personal history of breast cancer who received treatment at participating research facilities and had hormone receptor positive breast cancer, Oncotype DX testing and hereditary cancer mutation testing were included. Oncotype DX RS was recorded along with the type of genetic testing and the genetic testing results. RS was categorized as low (0-17), intermediate (18-30), and high (31+). Those with deleterious mutations in any known hereditary cancer gene were considered positive. Individuals with a variant of uncertain significance (VUS) or negative genetic testing result were considered negative. Difference in distribution of tumors with low, intermediate, and high Oncotype DX results in those with hereditary breast cancers compared to those with sporadic breast cancers was determined with Chi-square.

Results
419 patients with Oncotype DX testing from two clinical sites were collected from 2013. Of those, 123 underwent genetic risk assessment. Mutations identified included the following genes: BRCA1 (1), BRCA2 (5); CHEK2 (3); BRIP1 (3); NBN (2); MSH6 (1). Of those testing positive for a deleterious mutation, the number of patients with RS results in each category were 5, 4 and 6 for low, intermediate and high, respectively. For those considered negative on hereditary cancer panel testing, the RS results were 76, 52 and 8, respectively. Of those with high RS, 43% had deleterious mutations. Chi square test was statistically significant for a difference between the RS of those with deleterious hereditary mutations versus those with sporadic cancers (p = 0.000086).

Conclusions
High RS may indicate a higher likelihood of harboring a hereditary cancer syndrome. Further investigation with larger numbers and multivariate analysis is needed to validate if a high RS serves as an independent predictor of benefit from genetic counseling and testing.
Title: Androgen receptor positivity is associated with nodal disease in triple negative breast cancer


1The University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Background: Gene expression profiling (GEP) has identified several molecularly distinct subtypes of triple negative breast cancer (TNBC). Currently, GEP-based molecular diagnostics are not routinely used in clinical decision making due to the lack of proven benefit, costs involved and long turnaround time. However, two molecularly distinct subtypes of TNBC, the luminal androgen receptor (AR) and mesenchymal subtypes, have surrogate CLIA-certified immunohistochemical (IHC) markers, AR and vimentin (VM), respectively, which have the potential for application in the clinic. Here we report the rates of AR and VM positivity and their association with clinicopathological characteristics in a cohort of TNBC pts receiving NACT.

Methods: As part of an ongoing molecular triaging protocol, 144 pts with stage I-III TNBC underwent a pretreatment biopsy for molecular characterization (MC) prior to initiating neoadjuvant chemotherapy (NACT). IHC for AR and VM were performed using commercially available antibodies. AR+ and VM+ were defined as ≥10% and ≥50% staining, respectively. Pts were randomized 2:1 to know (intervention arm, n=93) and not know (control arm, n=51) the MC results. The charts of pts randomized to the intervention arm were reviewed. Categorical variables were analyzed using Fisher's exact test. Ordinal and continuous variables were analyzed using the Wilcoxon rank-sum test and Student's t test as appropriate.

Results: 31% (29/93) and 16% (15/93) of pts were AR+ and VM+, respectively. Only 4% (4/93) of pts were both AR+ and VM+. Clinicopathological characteristics are summarized in Table 1. AR+ pts were more likely to have clinically node positive disease as compared to AR- pts (66% vs 34%, p=0.007). There were no significant differences in clinical tumor size or grade between AR+ and AR- pts. VM+ and VM- pts had similar clinicopathological characteristics.

Conclusion: Pts with AR+ TNBC were more likely to have node positive disease. The impact of AR+ on long term outcomes should be investigated in prospective studies.

Table 1: Association between patient characteristics and AR/VM status

<table>
<thead>
<tr>
<th></th>
<th>AR</th>
<th>VM</th>
<th>p-value</th>
<th>AR</th>
<th>VM</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age - Median (years, interquartile range)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>AR+ (n=29)</td>
<td>58 (48-65)</td>
<td>52 (46-61)</td>
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<td>55 (48-64)</td>
<td>56 (47-62)</td>
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<tr>
<td><strong>Clinical Tumor Size</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (cm, standard deviation)</td>
<td>3.5 (1.8)</td>
<td>3.0 (1.8)</td>
<td>0.287</td>
<td>2.7 (1.7)</td>
<td>3.3 (1.9)</td>
<td>0.31</td>
</tr>
<tr>
<td>T1 – n(%)</td>
<td>5 (17)</td>
<td>21 (33)</td>
<td>0.230</td>
<td>7 (47)</td>
<td>19 (24)</td>
<td>0.098</td>
</tr>
<tr>
<td>T2 – n(%)</td>
<td>21 (72)</td>
<td>36 (56)</td>
<td></td>
<td>7 (47)</td>
<td>50 (64)</td>
<td></td>
</tr>
<tr>
<td>T3 – n(%)</td>
<td>3 (10)</td>
<td>7 (11)</td>
<td></td>
<td>1 (7)</td>
<td>9 (12)</td>
<td></td>
</tr>
<tr>
<td><strong>Clinical Nodal Status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative – n(%)</td>
<td>10 (34)</td>
<td>42 (66)</td>
<td>0.007</td>
<td>8 (53)</td>
<td>44 (56)</td>
<td>1.00</td>
</tr>
<tr>
<td>Positive – n(%)</td>
<td>19 (66)</td>
<td>22 (34)</td>
<td></td>
<td>7 (47)</td>
<td>34 (44)</td>
<td></td>
</tr>
<tr>
<td><strong>Grade</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 – n(%)</td>
<td>6 (21)</td>
<td>5 (8)</td>
<td>0.076</td>
<td>3 (20)</td>
<td>8 (10)</td>
<td>0.29</td>
</tr>
<tr>
<td>3 – n(%)</td>
<td>23 (79)</td>
<td>59 (92)</td>
<td></td>
<td>12 (80)</td>
<td>70 (90)</td>
<td></td>
</tr>
</tbody>
</table>
The quality and quantity of visceral fat tissue are associated with insulin resistance and survival outcome after chemotherapy for patients with early-stage breast cancer

Toshiaki Iwase¹, Takafumi Sangai¹, Takeshi Nagashima¹, Masahiro Sakakibara¹, Hiroshi Fujimoto¹, Yuji Sawabe³, Kengo Nagashima² and Masayuki Otsuka¹. ¹Chiba University Hospital, Chiba, Japan; ²Department of Global Clinical Research/Biostatistics, Chiba, Japan and ³Department of Laboratory Medicine, Chiba, Japan.

Body: Background: Obesity and insulin resistance are associated with inferior levels of chemosensitivity and overall prognosis for breast cancer (BC) treatment. Recent studies suggest that the quality and quantity of visceral adipose tissue (VAT) play a significant role in adipocyte function, and are related to insulin resistance. We therefore tested the hypothesis that high amount and low quality of VAT worsen treatment outcomes via insulin resistance mechanisms.

Patients and Methods: We examined two independent studies: a cross-sectional study (cohort 1) and a retrospective study (cohort 2). Cohort 1 included 106 women with early-stage BC who were undergoing surgery. Patients with normal weight (17.5< body mass index [BMI, kg/m²] ≤25, n = 53) and overweight/obese patients (BMI >25, n = 53) were selected by a pair-matching method. Insulin resistance was evaluated by HOMA-R: fasting insulin (microU/L) × fasting glucose (nmol/L)/22.5. And insulin-like growth factor (IGF) family including IGF-1 and IGF-binding protein 3 (IGFBP3) were measured before beginning treatment. The amounts of visceral fat (aVAT) was measured by 3-dimensional volumetric software using the stocked computed tomography (CT) imaging data. The quality of VAT was assessed based on the mode value of CT Hounsfield Unit of VAT (VAT-HU) at navel level of CT axial view. The association between the former variables and the quality and quantity of VAT was analyzed. Cohort 2 included 271 patients who received chemotherapy in the neo-adjuvant (NAC) or adjuvant setting. Imaging analysis was performed in the same way, and the association between those values and survival outcome after chemotherapy was analyzed by retrospective chart review.

Results: In cohort 1, aVAT was significantly correlated with serum insulin and HOMA-R levels (Pearson's R 0.44 and 0.42, respectively; P<0.05). On comparing the two groups divided by BMI, the levels of IGF-1 and IGFBP3 were not significantly different between the normal weight and the overweight/obese groups (P = 0.31 and 0.77, respectively). However, the overweight/obese group demonstrated significantly higher HOMA-R (P<0.05). In cohort 2, aVAT was significantly correlated with BMI (P<0.05). In a multivariate analysis, pathological complete responses were not associated with aVAT (P = 0.60). After a median follow-up of 112 months, tertile stratification revealed that the third tertile of aVAT had a significantly shorter distant disease free survival (DDFS) in the NAC setting (p<0.05). When adjusted by covariates in the Cox proportional regression model, aVAT and VAT-HU demonstrated significant contribution to a worsened DDFS (p<0.05, hazard ratio (HR) 1.39; 95% confidence interval (CI) 1.11 to 1.75) and (p<0.05, HR 1.20, 95% CI 1.01 to 1.43), respectively.

Conclusions: Our study found that high amounts and low quality of VAT worsen treatment outcomes. Furthermore, we found that insulin resistance was related to those two factors. Although further validation is needed, our present work suggests the importance of evaluating the quality and quantity of visceral fat for estimating insulin resistance and treatment outcomes after chemotherapy for patients with early-stage BC.
Title: Evaluation of the prognostic value of all four HER family receptors in patients with metastatic breast cancer treated with trastuzumab: A hellenic cooperative oncology group (HeCOG) study

Body: Background-aim: Metastatic breast cancer (MBC) is an incurable disease. Trastuzumab, a recombinant humanized monoclonal antibody, was found to significantly prolong survival of patients with metastatic HER2 over-expressing and/or amplified breast cancer. In the current study, we performed a complete analysis, with four different methods, of all four HER family receptors, in a series of patients with metastatic breast cancer treated with trastuzumab-based regimens and evaluated their prognostic value.

Methods: Formalin-fixed paraffin-embedded tumor tissue samples were collected from 227 patients, considered to be HER2-positive when assessed at the local laboratories. We evaluated gene amplification, copy number variations (CNVs), transcriptional profiling and protein expression of all four HER family members. In addition, our analysis included the evaluation of several other factors by immunohistochemistry (IHC), such as pHER2Tyr1221/1222, pHER2Tyr877 and PTEN.

Results: Central review of HER2 status by IHC and fluorescence in situ hybridization revealed that of the 227 patients, only 139 (61.2%) were truly HER2-positive. Regarding the 191 patients treated with trastuzumab as first-line therapy, median time to progression (TTP) was 15.3 months and 10.4 months for HER2-positive and HER2-negative participants, respectively. Median survival was 50.4 months for HER2-positive and 38.1 months for HER2-negative patients. In HER2-positive patients, high HER3 mRNA expression was of favorable prognostic significance for TTP and survival (HR=0.43, 95% CI 0.21-0.88, Wald's p=0.022 and HR=0.43, 95% CI 0.21-0.88, p=0.021, respectively), while EGFR copy gain and EGFR protein expression were associated with higher risk for disease progression in HER2-negative patients (HR=3.53, 95% CI 1.19-10.50, p=0.023 and HR=3.37, 95% CI 1.12-10.17, p=0.031, respectively). Positive HER3 protein expression was a favorable factor for TTP in HER2-negative patients (HR=0.43, 95% CI 0.22-0.84, p=0.014). In the multivariate analysis, only EGFR copy gain retained its prognostic significance for TTP in the HER2-negative population (HR=4.81, 95% CI 1.52-15.82, p=0.008), while none of the examined factors retained their prognostic significance for TTP or survival in the HER2-positive subgroup.

Conclusions: The present study suggests that EGFR copy gain represents a negative prognostic factor for TTP in HER2-negative patients with metastatic breast cancer. In addition, high HER3 mRNA expression appears to be of favorable prognostic significance for TTP and survival in HER2-positive patients. Given the small number of patients included in the current analysis and the retrospective nature of the study, our findings should be validated in larger cohorts.
2017 San Antonio Breast Cancer Symposium

Publication Number: P1-07-25

Title: Differences among young breast cancer patients based on subtype: A study from the Korean Breast Cancer Society – Running head: Do breast cancers in 20s have worse prognosis than 30s?

Jai Min Ryu¹, Jonghan Yu¹, Seok Jin Nam¹, Isaac Kim¹, Jeong Eon Lee¹, Se Kyung Lee¹, Jae Myung Kim¹, Hee Jun Choi¹ and Seok Won Kim¹. ¹Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea.

Body: Purpose Numerous studies demonstrated that breast cancer in young women (BCY) has unfavorable prognostic features and unfavorable subtype. However, there were few studies to evaluate the effect on the prognosis of breast cancer according to the subtype disparities by age especially BCY. We analyzed breast cancer mortality stratified tumor subtype according to age among the patients with less than 50 year-old. Patients and Methods Data obtained from the Korean Breast Cancer Society Registry (KBCSR), patients diagnosed with invasive breast cancer were retrospectively between 2003 and 2010. We excluded patients with male breast cancer, underwent neoadjuvant chemotherapy, distant metastasis or inflammatory breast cancer at presentation, and other histopathology except invasive ductal or invasive lobular carcinoma. We also excluded patients with lack of immunohistochemistry data and short-term follow-up duration (<12 months). Results We identified 37,865 patients, and excluded by study protocol. Among those, 30,793 patients with breast cancer for eligible for analysis, 793 (2.6%) were 20-29 years and 8,926 (28.8%) were 30-39 years of age. Median follow-up duration was 84 months. Mean age was 42.4 years old. Younger patients with breast cancer were more likely to have advanced stage, higher nuclear grade, present lymphovascular invasion, and more likely to be unfavorable subtype such as triple negative breast cancer (TNBC)

Table 1. Baseline Characteristics

<table>
<thead>
<tr>
<th>Age at Presentation</th>
<th>20-29, N(%)</th>
<th>30-39, N(%)</th>
<th>40-49, N(%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>793 (2.6)</td>
<td>8,133 (26.4)</td>
<td>21,867 (71.0)</td>
<td></td>
</tr>
<tr>
<td>Pathologic stage</td>
<td></td>
<td></td>
<td></td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>I</td>
<td>295 (37.2)</td>
<td>2,928 (36.0)</td>
<td>9,288 (42.5)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>373 (47.0)</td>
<td>3,644 (44.8)</td>
<td>9,078 (41.5)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>119 (15.0)</td>
<td>1,442 (17.7)</td>
<td>3,211 (14.7)</td>
<td></td>
</tr>
<tr>
<td>Family history</td>
<td>&lt; .0001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>81 (10.2)</td>
<td>674 (8.3)</td>
<td>1,391 (6.4)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>712 (89.8)</td>
<td>7,459 (91.7)</td>
<td>20,476 (93.6)</td>
<td></td>
</tr>
<tr>
<td>Nuclear grade</td>
<td>&lt; .0001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>85 (10.2)</td>
<td>941 (11.6)</td>
<td>3,824 (17.5)</td>
<td></td>
</tr>
<tr>
<td>Intermediate</td>
<td>288 (36.3)</td>
<td>3,340 (41.1)</td>
<td>9,688 (44.3)</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>331 (41.7)</td>
<td>3,165 (38.9)</td>
<td>6,650 (30.4)</td>
<td></td>
</tr>
<tr>
<td>LVI</td>
<td>&lt; .0001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>249 (31.4)</td>
<td>2,840 (34.9)</td>
<td>6,711 (30.7)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>433 (54.6)</td>
<td>4,367 (53.7)</td>
<td>13,005 (59.5)</td>
<td></td>
</tr>
<tr>
<td>Subtype</td>
<td>&lt; .0001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luminal A</td>
<td>314 (39.6)</td>
<td>3,529 (43.4)</td>
<td>11,716 (53.6)</td>
<td></td>
</tr>
<tr>
<td>Luminal B</td>
<td>190 (24.0)</td>
<td>1,895 (23.3)</td>
<td>4,775 (21.8)</td>
<td></td>
</tr>
<tr>
<td>Her-2</td>
<td>52 (6.6)</td>
<td>724 (8.9)</td>
<td>1,723 (7.9)</td>
<td></td>
</tr>
<tr>
<td>TNBC</td>
<td>237 (29.8)</td>
<td>1,895 (24.4)</td>
<td>3,653 (16.7)</td>
<td></td>
</tr>
</tbody>
</table>
Patients with younger age group showed worse prognosis than patients with older age patients. In multivariate analysis for overall survival, as patients were younger group, hazard ratio was increased, and the patients with TNBC showed higher HR than HER-2, Luminal B, and Luminal A subtype ($P < .0001$, $P < .0001$, $P < .0001$, and $P < .0001$, respectively). Stratified by subtype, luminal subtype showed significant worse prognosis as the age group was younger, while as, HER-2 and TNBC subtype showed no significantly different by the age group. **Conclusion** Patients with 20s breast cancer showed unfavorable characteristics and worse prognosis than 30s and older aged group. Stratified by tumor subtype, breast cancer in 20s with luminal subtype showed worse prognosis, while as HER-2 and TNBC showed no significantly different compare to breast cancer in 30s.
Clinicopathological predictors of axillary lymph node metastasis in early breast cancer patients

Mohan Satish¹, Ryan Walters¹ and Peter T Silberstein¹. ¹Creighton University School of Medicine, Omaha, NE.

Axillary lymph node (ALN) status is key in the prognosis of early breast cancer. Sentinel lymph node biopsy (SLNB) is the standard treatment in determining ALN status in clinically node negative patients with early breast cancer, but nearly 70% exhibit no ALN metastasis (a regional lymph node (RLN) metastasis). Likewise, selective use of SLNB has engendered modeling ALN metastasis. Using the National Cancer Database (NCDB), this study aimed to evaluate clinicopathological factors to help predict first and subsequent RLN metastasis in patients with early breast cancer.

Methods: We identified 660,258 women from 2004-2013 with early breast cancer stage (cT1-T3, cN0-N1, and M0). A two-part model was estimated using multivariable logistic regression to evaluate 1) predictors, by odds (OR), of having at least one RLN metastasis, and 2) predictors, by rate difference (RD), of having additional RLN metastasis relative to women with only one RLN metastasis. The same set of predictor variables were included in both parts of the model. All analyses were conducted using SAS v. 9.4 with p <.05 indicating statistical significance.

Results: Adjusted ORs and RDs of RLN metastasis for selected variables from the model are shown in Table 1. Increased likelihood of at least one RLN metastasis was significantly associated with the presence of larger tumor size (p <0.0001), a primary tumor in the nipple region (p <0.0001) of the left breast (p <0.0001), lobular or ductal type histology (p <0.001, and p <0.0001, respectively), positive estrogen (ER) and progesterone (PR) receptor statuses (p <0.0001 for both), younger age (p <0.0001), being white (p <0.0001), and greater comorbidity (Charlson/Deyo - CD) (p < 0.0001). Predictors of at least one RLN metastasis were also significantly associated with higher adjusted rates of further metastasis, except age, tumor size, ductal type histology, ER, and PR. However, a primary tumor in the central region (p =0.037), not the nipple region, was most associated with additional metastasis.

<table>
<thead>
<tr>
<th></th>
<th>≥1 event</th>
<th>&gt;1 event</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (continuous)</td>
<td>0.99 (0.99-0.99)</td>
<td>1.00 (-)</td>
</tr>
<tr>
<td>White vs. Other</td>
<td>1.10 (1.05-1.14)</td>
<td>1.02 (1.00-1.04)</td>
</tr>
<tr>
<td>CD = 0 vs. 1</td>
<td>0.98 (0.97-0.99)</td>
<td>0.97 (0.95-0.99)</td>
</tr>
<tr>
<td>CD = 0 vs. ≥2</td>
<td>1.02 (1.02-1.02)</td>
<td>1.00 (-)</td>
</tr>
<tr>
<td>Tumor Size (continuous)</td>
<td>1.00 (-)</td>
<td></td>
</tr>
<tr>
<td>Primary Tumor Site - Nipple vs. (left), Central vs. (right)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overlap</td>
<td>1.80 (1.61-2.02)</td>
<td>1.02 (1.01-1.04)</td>
</tr>
<tr>
<td>Tail</td>
<td>1.87 (1.59-2.20)</td>
<td>0.98 (-)</td>
</tr>
<tr>
<td>LOQ</td>
<td>1.64 (1.46-1.84)</td>
<td>1.00 (-)</td>
</tr>
<tr>
<td>UOQ</td>
<td>1.71 (1.53-1.92)</td>
<td>1.02 (1.00-1.03)</td>
</tr>
<tr>
<td>LIQ</td>
<td>2.07 (1.84-2.33)</td>
<td>1.07 (1.04-1.09)</td>
</tr>
<tr>
<td>UIQ</td>
<td>2.73 (2.43 -3.07)</td>
<td>1.11 (1.09-1.13)</td>
</tr>
<tr>
<td>Central (left), Nipple (right)</td>
<td>1.44 (1.28 -1.63)</td>
<td>1.06 (1.02-1.11)</td>
</tr>
<tr>
<td>Histological Type - Lobular vs. Other</td>
<td>4.00 (3.72-4.30)</td>
<td>1.52 (1.46-1.57)</td>
</tr>
<tr>
<td>Ductal</td>
<td>0.99 (-)</td>
<td>1.18 (1.17-1.19)</td>
</tr>
<tr>
<td>ER (Positive vs. Negative)</td>
<td>1.04 (1.04-1.04)</td>
<td>1.00 (-)</td>
</tr>
<tr>
<td>PR (Positive vs. Negative)</td>
<td>1.02 (1.02-1.02)</td>
<td>1.00 (-)</td>
</tr>
</tbody>
</table>
Conclusion: Utilizing a large dataset, several clinicopathological factors emerged from the NCDB as independent predictors of at least one, or additional RLN metastasis, supporting their weighted inclusion in prediction tools for ALN metastasis. Notably, different primary tumor sites of the breast predicted the two events modeled.
**Title:** Prognostic value of programmed death 1/Programmed death ligand 1/ mammalian target of rapamycin/Rictor/Tuberin in human breast cancer

Michal Uhercik¹,³, Andrew J Sanders¹, Sioned Owen¹, Eleri L Davies², Anup K Sharma³, Wen G Jiang¹ and Kefah Mokbel⁴.

¹Cardiff China Medical Research Collaborative at Cardiff University, Cardiff, United Kingdom; ²Cardiff Breast Centre, University Hospital Llandough, Cardiff and Vale University Health Board, Cardiff, United Kingdom; ³St George’s University Hospital, London, United Kingdom and ⁴The London Breast Institute, Princess Grace Hospital, London, United Kingdom.

**Body:**

**Background:** The Mammalian Target of Rapamycin (mTOR) regulates a multitude of cellular processes including metabolism, proliferation and growth. It is known to form two multi-protein complexes - complex 1 (mTORC1) and complex 2 (mTORC2) with Raptor and Rictor being their core proteins vital for their integrity.

Tuberin, the product of the Tuberous Sclerosis Complex gene 2, TSC2, has been characterized as a tumour suppressor and negatively regulates the mTOR pathway.

Programmed Death 1 (PD-1), a transmembrane protein particularly expressed on the surface of tumour cells, acts as an immune checkpoint receptor. Together with its ligand Programmed Death Ligand 1 (PDL-1) they form a pathway which when activated influences anti-tumour immunity and suppresses anti-tumour adaptive responses. The expression of PD1/PDL-1 is lightly regulated by the mTOR pathway.

We investigated the value of expression patterns of all these molecules in breast cancer as potential prognostic factors.

**Materials and Methods:** Quantitative PCR (qPCR) analysis was used to determine the transcript expression profile of the five genes of interest (PD-1, PDL-1, mTOR, Rictor and Tuberin) in 128 breast cancer specimens. The correlation between PD-1 or PDL-1 with mTOR, Rictor and Tuberin was assessed using the Spearman Rank Order Correlation. Subsequently, a combined analysis was performed, where the influence of favourable expression in relation to patient overall (OS) and disease free survival (DFS) using the Kaplan Meier survival curves and multivariate analysis.

**Results:** The mRNA expression of the molecules showed a varying degree of association with the clinicopathological parameters. PD-1 transcript expression showed a significant correlation with mTOR expression (p < 0.001). PDL-1 transcript expression was seen to correlate with mTOR (p < 0.001), Rictor (p < 0.001) and Tuberin (p < 0.01) transcript expression.

However, when the expression profile was analysed using an integrated expression score, the combined predictive value for the clinical outcome of the five genes was highly significant in terms of OS (p < 0.001) and DFS (p = 0.001), and was found to be an independent prognostic factor (p<0.001) for breast cancer related death using a multivariate analysis.

**Conclusions:** Our study identifies a molecular signature of 5 genes as a powerful prognostic predictor of OS and DFS in patients with breast cancer.
Title: Retrospective analysis of clinicopathologic features predictive of oncoType DX discordance in estrogen receptor positive, node negative breast cancer patients

Krystal P Cascetta¹, Brittney S Zimmerman¹, Lauren Eggert², Madeline C Molot³, Meng Ru¹, Anupma Nayak⁴, Ira Bleiweiss⁴ and Amy Tiersten¹. ¹Mount Sinai Hospital and Icahn School of Medicine, New York, NY; ²Stanford School of Medicine, Stanford, CA; ³Barnard College of Columbia University, New York, NY and ⁴Hospital of the University of Pennsylvania, Philadelphia, PA.

Body: BACKGROUND: Oncotype DX (ODX) is a validated recurrence score (RS) used to predict the risk of recurrence and benefit of chemotherapy in ER positive, node negative breast cancer patients. Prior to ODX, treatment recommendations regarding adjuvant chemotherapy and mortality approximation have taken into account clinical and pathologic risk factors. A discordance rate of 7-19% between risk allocating pathologic factors and ODX RS has been previously reported with progesterone receptor (PR) negativity noted as a defining clinical feature in numerous cases. The association between other clinicopathologic features and discordance is less certain.

METHODS: ODX data and clinicopathologic features were retrospectively reviewed for 724 breast cancer tumors belonging to 704 patients between 2006 and 2016. ODX discordance was defined as either 1-step discordance or 2-step discordance between ODX risk group (low, intermediate, high) and tumor grade (TG) (well differentiated, moderately differentiated, poorly differentiated). Tumors with 1-step discordance received a discordance score (DS) of 1 while those with 2-step discordance received a DS of 2. The database was subsequently analyzed using Paik's RS cutoffs as well as those outlined in the TAILORx trial. An odds ratio (OR) of >1 was consistent with discordance.

RESULTS: Among 724 tumor samples, ODX ER score (p=0.000), ODX PR score (p=0.000), ODX HER2 score (p=0.000), TG (p=0.000), mitotic count (MC) (p=0.0012), DCIS grade (p=0.0046), DCIS type (comedo necrosis vs. non-comedo necrosis) (p=0.0335) and micropapillary features (p=0.0044) were significantly associated with RS. Median age of cohort was 59 years and median tumor size was 1.2 cm.

Of 724 tumors, 619 from 604 subjects were eligible for assessment of discordance. Median RS was 16. Using Paik's RS cutoffs, 64.3% discordance was observed: 52.5% 1-step discordance (DS 1) and 11.8% 2-step discordance (DS 2). The TAILORx categorization yielded a discordance rate of 44.3%: 40.1% 1-step discordance and 4.2% 2-step discordance. On univariate analysis and using Paik's RS cutoffs, young age (p= 0.0240), high MC (p=0.0006), large tumor size (>20 mm) (p=0.0209), the presence of DCIS (p=0.0480), high DCIS grade (p= 0.0033), and high ODX PR and ER scores (p= 0.0000) were significant clinicopathologic features predictive of discordance. On multivariate analysis, high MC (p= 0.0000), high ODX PR and ER scores (p=0.0000) remained significant as well as premenopausal status (p=0.026).

CONCLUSION: In this retrospective ODX database, premenopausal status, high MC, high ODX PR and ER scores as per Paik's RS cutoffs were significant predictors for ODX discordance while high ODX PR and ER scores were significant predictors per the RS's outlined in the TAILORx trial. RS cutoffs per the TAILORx trial appear to create less discordance between RS and TG than the original cutoffs outlined by Paik and colleagues.
Clinicopathologic characteristics and prognostic factors of pure mucinous breast cancer

Sung Chan Gwark, Jong Won Lee, Sae Byul Lee, Guiyun Sohn, Jisun Kim, Il Yong Chung, Hee Jeong Kim, Beom Seok Ko, Byung Ho Son, and Sei Hyun Ahn. Asan Medical Center, Seoul, Republic of Korea.

Background: Mucinous carcinoma of the breast is a uncommon particular type of breast cancer and comprises approximately 4% of all invasive breast cancers. It is characterized by abundant extracellular mucin production and present a more favorable prognosis than IDC-NOS. Pathologically, mucinous carcinoma is divided into two subtypes: pure and mixed. In this study, we reviewed the clinicopathologic characteristics and prognostic factors of pure mucinous carcinoma.

Methods: We reviewed the 23 years cumulative data of pure mucinous breast cancer patients from database of the Breast Cancer Center at ASAN medical center, Korea, between 1989-2011, retrospectively. Total 386 pure mucinous carcinoma cases were reviewed to analyze clinicopathologic characteristics and prognosis.

Result: Total of 386 patients with pure mucinous breast cancer were identified. Mean age was 46.7. 149 patients underwent modified radical mastectomy and 236 underwent breast-conserving therapy. The T-stage was T1 in 187 patients, T2 in 178 patients, T3 in 17 patients and T4 in 4 patients. Node negative was 325 and node positive was 61. Estrogen receptor was positive in 342 and negative in 29. Progesterone receptor was positive in 342 and negative in 29. Progesterone receptor was positive in 276 and negative in 95. HER-2 was positive in 47 and negative in 273. 152 patients received adjuvant chemotherapy and 240 patients received adjuvant radiotherapy. 351 patients received hormone therapy and among these patients, 231 patients were treated with Tamoxifen alone, 81 were Tamoxifen with ovarian function suppression, 37 were AI only and one patient was ovarian function suppression only. The 5 year disease free survival rate was 93.3%, 5 year cancer specific survival rate was 98.2% and 5 year overall survival rate was 96.6%. Univariate analysis showed that ER status, nodal status and Her2 status were appear to be prognostic factor of Disease free survival rate. Using Cox regression, result of multivariate analysis revealed that only nodal status is the most significant prognostic factor for survival rate.

Conclusions: Pure mucinous carcinoma of the breast is a rare subtype with a favorable prognosis. Nodal status rather than ER status, Her2 status are considered to be the most significant prognostic factor of pure mucinos breast cancer.
Title: Predictive factors of sentinel lymph node biopsy invasion in extended ductal carcinoma in situ treated by radical mastectomy

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Body: Introduction: The incidence of ductal carcinoma in situ (DCIS) increased with the practice of organized breast cancer screening to reach 14 % in France in 2010. The incidence of positive sentinel lymph node biopsy (SLNB) ranged from 0 to 16.7 %. The main hypothesis would be the presence of an invasive contingent on the definitive analyze not identified preoperatively. The objective was to identify predictive factors of SLNB positivity in the management of extended DCIS treated by radical mastectomy.

Method: This study was retrospective, longitudinal, descriptive conducted at the Cancer Institute of Lorraine from January 2000 to July 2015. All patients whose management consisted of a radical mastectomy for an extended DCIS, associated with a sentinel lymph node procedure were included.

Results: 161 patients were included. The mean age at diagnosis was 56 years; 15 had a clinical nodule. The diagnosis was made in 63.3 % with macrobiopsies. Preoperatively, 16 patients (9.9 %) had DCIS associated with microinvasion (DCIS-MI) and the others were pure DCIS. An average of 3.9 ± 2.7 SLNB were sampled. Twelve patients (7.4 %) had a SLNB invasion. Eleven of them had axillary lymph node dissection (ALND) of which only 1 was positive. The final histological analysis found 104 pure DCIS (64.6 %), 23 DCIS-MI (14.3 %) and 34 occult invasive ductal carcinomas (IDC) (21.1 %). Mean follow-up was 41.1 months. There were 2 recurrences and 2 deaths.

<table>
<thead>
<tr>
<th>No</th>
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<th>DCIS size (mm)</th>
<th>Palpability</th>
<th>SLNB type</th>
<th>SLNB positivity</th>
<th>ALND positivity</th>
</tr>
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<tbody>
<tr>
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</tr>
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<td>-</td>
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<td>0</td>
</tr>
<tr>
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<td>0</td>
</tr>
<tr>
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<td>No</td>
<td>Isolated Tumor Cells</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
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<td>80</td>
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</tbody>
</table>

Predictive factors were size of palpated mass (mean: 46 mm, p = 0.04) and microinvasion on biopsy (p = 0.02). Positivity of the SLNB was an overstaging risk factor on the final histology (p < 0.001). Postoperative histological results were significantly different from preoperative (p = 0.001) with poor concordance (kappa = 0.15).

Discussion: After SLNB, the rate of secondary lymphedema in the literature was 5%. Our study included 7.4 % (12 cases) of axillary lesions, majority of which were unique micrometastases or isolated tumor cells (ITC). All predictive factors were identified in literature. In our study, mean size of palpated mass was 46 mm whereas it was 30 mm in literature. Of the 9.9 % of DCIS-MI, 4 patients had a positive SLNB. In cases of pure DCIS, the percentage of positive SLNB was reduced to 5.5 %. The rate of occult invasive ductal carcinoma was 21,1%, similarly like in literature where rate was in mean 23%.
Conclusion: The low rate of SLNB invasion in pure DCIS suggests that ALND is carried out in the presence of predictive factors. New techniques for identification of SLNB could report axillary staging after obtaining the definitive histologic results.
Body: Backgrounds: MicroRNA (miRNA) play a crucial role in cancer progression, and altered miRNA expression has been demonstrated to be associated with breast cancer. MiRNA is also known to be stably detectable not only in tissue but peripheral circulation; therefore it could be a feasible and potential biomarker of breast cancer in clinical settings. However, few studies have been reported to identify promising miRNA profiles as predictive biomarker using statistically satisfied large cohorts of breast cancer patients. The aim of this study is to identify miRNA signature that can predict patient survival utilizing integrated and unbiased transcriptomics analyses.

Methods: Integrated and unbiased transcriptomics approach was conducted on genomic and clinicopathological information of 2580 breast cancer patients. We utilized The cancer Genome Atlas (TCGA) to identify miRNA signature that could significantly associated with clinical relevance including prognosis and metastatic information, followed by validation analyses with the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) and Gene Expression Omnibus (GEO)

Results: A novel risk scoring model including three miRNAs signature (miR-19a, miR-93, and miR-106a) was identified using Cox model. This miRNA score was able to extract the patient population with extremely poor prognosis in TCGA (5-yr overall survival rate, 49.2 %, p=0.0005). This result was validated with another three completely independent cohorts with microarray dataset accompanied with sufficient clinical information and miRNA expression (GSE19536, n=96, p=0.0009; GSE22220, n=210, p=0.0003; METABRIC, n=1223, p=0.0023). Interestingly, the subtype stratification with PAM-50 classification using bioinformatics pipeline demonstrated that this miRNA score could predict poor overall or disease-free survival especially in the population with luminal A, B or normal like subtype (p=0.0300 and p=0.0001, respectively). In addition, competing risk analysis for tumor recurrences demonstrated that the risk scoring using three miRNAs signature could be significantly associated with bone metastasis (p=0.0052). Finally, Gene Set Enrichment Analysis (GSEA) identified that high risk score using three miRNAs associated significantly with several critical gene sets related to metastatic formation such as angiogenesis (p <0.0001), epithelial mesenchymal transition (EMT) (p = 0.0155), focal adhesion (p <0.0001), TGF-beta signaling pathway (p = 0.0025), and ECM receptor interaction (p = 0.0068).

Conclusions: We demonstrated a promising miRNAs signature score system for predicting extremely poor prognosis and metastatic potentiality in breast cancer using novel integrated transcriptomics concept.
Title: Validation of the new AJCC eighth edition of the TNM classification for breast cancer with a single center breast cancer cohort

Ji-Yeon Kim¹, Ji Eun Lim², Hae Hyun Jung², Soo Youn Cho¹, Eun Yoon Cho¹, Se Kyung Lee¹, Jong Han Yu¹, Jeong Eon Lee¹, Seok Won Kim¹, Seok Jin Nam¹, Yeon Hee Park¹, Jin Seok Ahn¹ and Young-Hyuck Im¹. ¹Samsung Medical Center, Seoul, Korea and ²Biomedical Research Institute, Seoul, Korea.

Body: Introduction : Our understanding of biology of breast cancer has led to significant changes in diagnostic and therapeutic approaches for breast cancer. The new eighth edition of the TNM classification of the AJCC for breast cancer (BC) determined by a multidisciplinary team of BC experts incorporate biologic factors, such as tumor grade, estrogen and progesterone receptor (ER and PR) expression, human epidermal growth factor 2 (HER2) expression, and gene expression prognostic panels in addition to traditional anatomic factors. In this study, we aimed to evaluate prognostic value of this new staging system compared to previous AJCC 7th staging system using single center, long term followed BC cohort.

Methods : We conducted a retrospective analysis of women with stage I, II, or III BC who underwent curative surgery with/without adjuvant systemic therapy at Samsung Medical Center between July 2004 and December 2008 (n=3,029). We excluded patients who received neoadjuvant therapy (n=183), and patients with missing information about immunohistochemistry (n=7), HER2 status (n=82) or histologic grade (n=74). The final sample size was 2,683.

Results: Of 2,683 BCs, 1,689 (63%) were hormone receptor (HR)-positive(+), 244(9%) were HR+ andHER2+, 289(11%) were HR-negative(-) andHER2+, 461(17%) were triple negative BCs. According to AJCC 7th pathologic staging system, there were 1,135 of stage IA, 4 of IB, 368 of stage IIA, 258 of stage IIIA, 11 of stage IIIB and 104 of stage IIIC. In terms of 10 year overall survival (OS) according to AJCC 7th staging system, 95.8% in stage IA, 100% in stage IB, 93.5% in IIA, 86.0% in stage IIB, 85.6% in stage IIIA, 90.9% in stage IIIB and 63.6% in stage IIIC were observed (p<0.001) (Medial follow up duration : 118 months). According to AJCC 8th clinical staging system, there were 722 of stage IA, 693 of IB, 306 of stage IIA, 201 of stage IIB, 251 of stage IIIA, 137 of stage IIIB and 160 of stage IIIC. In terms of 10 year overall survival (OS) according to new staging system, 96.7% in stage IA, 96.6% in stage IB, 91.4% in IIA, 92.1% in stage IIB, 86.2% in stage IIIA, 80.2% in stage IIIB and 66.8% in stage IIIC were observed (p<0.001). However, 213 BCs(7.9%) could not be staged by AJCC 8th clinical staging system. In depth analysis will be presented.

Conclusions: AJCC 8th clinical staging system provides a good prognostic value and makes up for the weakness of AJCC 7th anatomical pathologic staging. But this system cannot count whole pathologic stages. Modification of AJCC 8th clinical staging system would be warranted.
Impact of family history of breast and/or ovarian cancer in triple negative breast cancer

Zaida Morante1, Gabriel De la Cruz-Ku3, Joseph Pinto3, Daniel Enriquez1, Maria Lujan3, Renato Luque3, Eduardo Eyzaguirre3, Antonella Saavedra3, Hugo Fuentes1, Silvia Neciosup1 and Henry Gomez1. 1Instituto Nacional de Enfermedades Neoplasicas, Lima, Peru; 2Oncosalud, Lima, Peru and 3Universidad Cientifica del Sur, Lima, Peru.

Body: Background: A family history of breast and/or ovarian cancer (FHBOC) is a risk factor for breast cancer. The influence of FHBOC in the prognosis of triple-negative breast cancer (TNBC) is controversial. The aim of this study was to analyze the impact of FHBOC on the prognosis in TNBC patients.

Methods: We retrospectively reviewed a database of patients diagnosed of TNBC (period 2000-2014) and treated at “Instituto Nacional de Enfermedades Neoplasicas”. The patients were divided into two groups: with FHBOC and non-FHBOC. The clinical features and prognosis between groups were compared. Disease-free survival (DFS) and overall survival (OS) differences were calculated by the Kaplan-Meier Method and differences were evaluated by the Log-Rank test. Prognostic factors were identified by the Cox regression analysis.

Results: Overall, 266 out of 2007 TNBC patients, had +FHBOC (13.3%). The median age for the general population was 49 years. Compared with the non-FHBOC group (51.1%), the 43.8% +FHBOC were premenopausal. Prevalence of +FHBOC in a first-, second- and a third-degree relative was 26.8%, 28.5%, and 44.7%, respectively. Regarding histological subtype, invasive ductal carcinoma was the most prevalent (93.5%). Clinical stages (CS) II/III was more frequent in both groups (86.3 vs. 86.1). A total of 59% of FHBOC patients underwent surgery and 52% for non-FHBOC (p=0.029- Conservative 41.1 vs. 30.7 - Mastectomy 58.9 vs. 69.3). The median of follow-up was 9 years. DFS rate was higher in FHBOC patients (53.6% vs. 42.9%, p=0.001). OS was significantly better in the FHBOC group (Table 1). In the multivariate analysis, poor prognosis variables to DFS were an advanced age at diagnosis (≥50 years) (p=0.006) and higher clinical stage (CS I vs. CS III, p<0.001) while chemotherapy (CT) and radiotherapy (RT) were associated with an improved DFS. In regard to the OS, FHBOC was associated with a better prognosis (HR:0.77, 95%CI:0.62- 0.95, p=0.016).

Conclusion: Our results showed a better prognosis in TNBC patients with family history of breast and/or ovarian cancer in terms of DFS and OS.

Table 1. +FHBOC group (DFS/OS rates)

<table>
<thead>
<tr>
<th></th>
<th>+FHBOC</th>
<th>non-FHBOC</th>
<th>Total</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFS (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-years</td>
<td>71</td>
<td>62</td>
<td>63</td>
<td>0.032</td>
</tr>
<tr>
<td>10-years</td>
<td>63</td>
<td>50</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>OS (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-years</td>
<td>66</td>
<td>54</td>
<td>55</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>10-years</td>
<td>58</td>
<td>47</td>
<td>50</td>
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</table>
Title: Clinical outcomes of single versus double hormone receptor positive breast cancer patients treated with neoadjuvant chemotherapy

Jacques Raphael\textsuperscript{1,2}, Sharon Nofech-Mozes\textsuperscript{3} and Maureen E Trudeau\textsuperscript{2}. \textsuperscript{1}Institute of Health Policy, Management and Evaluation, University of Toronto, Toronto, ON, Canada; \textsuperscript{2}Sunnybrook Health Sciences Centre, Toronto, ON, Canada and \textsuperscript{3}Sunnybrook Health Sciences Centre, Toronto, ON, Canada.

Body: Purpose
This study aimed to evaluate and compare tumor response rates and survival outcomes between single and double hormone receptor (HR) positive (+) [Estrogen Receptor (ER+)/Progesterone Receptor (PR) negative (-) or ER-/PR+ versus ER+/PR+] breast cancer (BC) patients with any HER2 status treated with neoadjuvant chemotherapy at a single institution.

Methods
A retrospective review was conducted using the Sunnybrook “Biomatrix” database to identify eligible patients. A multivariable logistic regression analysis (MLR) was performed to assess the association between HR status (single or double HR+) and pathologic complete response (pCR) rates at surgery. A Kaplan-Meier method was used to estimate Disease Free Survival (DFS) and a log-rank test was used to compare DFS between 3 subgroups of patients: single or double HR+ and HR negative patients.

Results
Three hundred and four BC patients were identified and included in the analysis with a median follow up of 43.3 months (Q1-Q3: 28.7-61.1) and a mean age of 49.7 years (Standard deviation 10.9). Forty seven percent (47/101), 31% (11/36) and 14% (24/167) of patients with HR negative, single HR+ and double HR+ disease achieved a pCR respectively ($X^2$ test $<$0.0001). In a MLR analysis, HR status and HER2 status were associated with pCR rates. Compared to HR negative patients, patients with double HR+ disease were less likely to achieve pCR (Odd ratio (OR):0.14, 95%CI 0.06-0.31, $p$<0.0001) while single HR+ patients did not differ (OR:0.51, 95%CI 0.19-1.4). The association between HR+ status (single versus double HR+) and pCR rates compared to HR negative patients remained the same in subgroup analyses of HER2+ and HER2 negative patients separately. No difference in survival (DFS) was seen between the 3 subgroups of patients: HR negative, single and double HR+ patients.

Conclusion
BC patients with single HR+ disease behave differently than double HR+ patients in terms of likelihood of achieving pCR after neoadjuvant chemotherapy and do not differ from HR negative patients. This difference does not translate into a difference in DFS. Prospective studies are needed to validate these findings before considering different treatment strategies for these 2 subgroups of HR+ BC patients.
Adipokine receptor CAP1 expression as predictor of survival outcome among breast cancer patients

Ann H Rosendahl¹, Malin Bergqvist¹, Siker Kimbung¹ and Signe Borgquist¹². ¹Lund University, Clinical Sciences Lund, Oncology and Pathology, Lund, Sweden and ²Aarhus University, Aarhus, Denmark.

Background: Obesity is a challenging health concern in breast cancer. Not only are obese women at higher risk of breast cancer, but if diagnosed with the disease, obesity is also impairing the prognosis of a breast cancer. Understanding the underlying biology behind these associations are key to improve survival rates in breast cancer. In obesity, the more abundant, activated adipose tissue may lead to insulin resistance and secretion of soluble mediators that may contribute to impaired breast cancer outcomes observed among obese women. The adipokine resistin is implied to be an important link between obesity and insulin resistance, and to be elevated among breast cancer patients. Yet, the expression of the newly identified resistin receptor CAP1 and its impact on breast cancer prognosis is poorly understood.

Purpose: To evaluate the impact by the adipokine receptor CAP1 tumor expression on breast cancer outcomes.

Experimental Design: CAP1 mRNA expression was explored among 1,881 primary breast tumors. Associations between CAP1 expression, categorized into tertiles, and survival outcomes were estimated using the Kaplan-Meier method and log-rank test, and by multivariable Cox regression models providing adjusted hazard ratios (HRadj) with 95% confidence intervals (CI). Independent biological network interaction analyzes were conducted in a subset of 1,105 invasive breast carcinomas within The Cancer Genome Atlas (TCGA) project.

Results: CAP1 was linked to poor tumor characteristics with higher CAP1 expression found among estrogen receptor (ER)-negative tumors, relative to ER-positive tumors, as well as with increasing tumor grade (P=0.025). High CAP1 tumor expression was further associated with shorter overall survival (HRadj 1.54; 95% CI, 1.11-2.13) and relapse-free survival (HRadj 1.47; 95% CI, 1.10-1.96), compared with low or intermediate CAP1 expression, particularly among ER-positive tumors or lymph node positive tumors. Additionally, CAP1 was identified in complex with genes involved in growth promoting and cell motility processes.

Conclusion: These results highlight the potential role of the resistin receptor CAP1 regarding breast cancer outcomes. Further investigations are needed to elucidate the biological and clinical implication of the resistin-CAP1 link in obesity-associated breast cancer.
Title: Outcomes and failure of primary endocrine therapy for operable breast cancer in the elderly: A regional North-east of England study

Robert Thomas¹, Rachel Rowell², Siobhan Crichton³ and Henry Cain². ¹Gateshead NHS Foundation Trust, United Kingdom; ²Newcastle Upon Tyne Hospitals NHS Foundation Trust, United Kingdom and ³Medical Research Council Trials Unit University College London, United Kingdom.

Body: Introduction
Primary Endocrine therapy (PET) to treat oestrogen receptor (ER) positive operable breast cancer in the elderly is used widely in the UK. A previous Cochrane analysis revealed its inferiority in long-term local control in comparison to surgery. As such, current guidance from the Society of International Geriatric Oncologists (SIOG) suggest that PET should be reserved for patients with a reduced life-expectancy (2-3 years) or for those considered unfit for surgery. Inaccurate assessment of life expectancy could lead to treatment failure which is potentially catastrophic for the patient.

The aim of this study was to evaluate treatment failures in elderly breast cancer patients treated with PET and to determine predictors of failure.

Methods
A retrospective observational study was performed on consecutive patients with ER positive early stage breast cancer treated with PET between 2005 and 2015 in the three breast units in the north east of England. The primary outcome measure was treatment failure and the secondary outcome measure was disease progression.

Results
488 patients were included with mean follow up 31 months (SD 23). 465 (95.2%) patients were started on Letrozole as their initial therapy. Overall, 206 patients were still alive with their disease controlled at the end of follow up, 219 had died with their disease controlled and 63 (12%) experienced treatment failure. Kaplan-Meier survival analysis predicted at 3 years 40% of patients to have died with controlled disease and 10% to have failed, leaving 50% living beyond this point with controlled disease.

Regression analysis identified younger age [SHR 0.96 (95% CI 0.94 to 0.99) p 0.013], larger tumours [SHR 1.03 (1.01 to 1.06) p 0.015], grade 3 cancers [SHR 3.58 (1.93 to 6.63) p<0.001] and axillary lymph node metastases [SHR 1.93 (1.06 to 3.52) p 0.030] were all independent predictors of treatment failure. Disease progression was reported in 86 (17.6%) of patients.

Conclusions
This is the largest retrospective series conducted evaluating PET treatment failure. Our comparably low rate of failure in comparison to that in the literature (12-85%) reflects good clinical acumen. Clear predictors of failure have been identified, which support previous analyses, further validating our results. Patients are able to live longer term (beyond 3 years) and maintain local disease control, which may represent a viable long-term treatment in the absence of risk factors for failure. Further work on our series is underway examining the effect of decision-making on PET outcomes.
2017 San Antonio Breast Cancer Symposium

Publication Number: P1-08-01

Title: Combination checkpoint inhibition and epigenetic modulation promotes tumor suppression and improves survival in Her2+ models of breast cancer

Evanthia T Roussos Torres¹, Hayley Ma¹, Brian Christmas¹, Todd Armstrong¹ and Elizabeth M Jaffee¹. ¹Johns Hopkins University, Baltimore, MD.

Body: Background: Checkpoint inhibition is a very successful treatment strategy in cancers that are naturally immunogenic by attracting T cells into the tumor microenvironment (TME) and promoting cytotoxic signaling pathways. While this strategy has shown some efficacy in metastatic breast cancer, most breast cancers are not highly immunogenic likely due to an immunosuppressive microenvironment and a lack of tumor antigen expression and recognition. One strategy to transform the breast TME is to use epigenetic modulation to affect activation and trafficking of myeloid derived suppressor cells (MDSCs), known to alter the immunogenicity of the TME and sensitize tumors to checkpoint modulation. We hypothesize that combinatorial therapy primes the TME by altering infiltration and function of MDSCs leading to a more robust T cell response. Methods: We are using a HER-2/neu transgenic mouse model with tumor challenge of syngeneic cell lines to test the efficacy of different combinations of an epigenetic agent, the histone deacetylase inhibitor entinostat (ENT), checkpoint inhibitors anti-programmed cell death protein (a-PD-1) and anti-cytotoxic T-lymphocyte-associated protein 4 (a-CTLA-4) antibodies, with and without anti-HER2 antibodies. We will examine treatment effects on tumor growth, and hope to identify co-stimulatory and inhibitory factors regulating T cell and MDSC responses. Characterization of tumor infiltrating lymphocytes and their functional capabilities are being investigated in primary tumors using fluorescence-activated cell sorting, nanostring gene expression profiling, and immunohistochemistry. Results: We found significant improvement in survival and delay in tumor growth in mice treated with ENT in combination with a-PD-1 and/or a-CTLA-4. Addition of anti-HER2 therapy to ENT and a-CTLA4 or a-PD1 also significantly improves survival and delay in tumor growth. We also found addition of ENT to checkpoint inhibition leads to significantly increased infiltration of granulocytic-MDSCs into the TME. We demonstrate an increase in CD8+ T effector cells in mice treated with combination therapy. Flow cytometric evaluation of markers of T cell activation, exhaustion, and MDSC function demonstrate significantly increased T cell activation, exhaustion, and myeloid function however it is unclear how this directly effects the phenotype we have observed in these mice. Gene expression profiling of both MDSCs and lymphocytes infiltrating tumors is underway to help determine significant changes in immune related pathways that lead to our observed outcomes. Conclusions: Addition of ENT to checkpoint inhibition significantly increases infiltration of innate and adaptive immune cells into the highly tolerant neu-N breast tumors and leads to improved survival and decreased tumor burden. Functional assays are underway and future studies will further delineate changes in immune infiltration as well as genetic alterations responsible for these observations. It is our hope that these novel findings will provide further rationale for combination therapy and improve the response rate of these immune therapies in patients with breast cancer.
Title: Breast tumor-specific MHC-II expression drives a unique pattern of adaptive resistance to antitumor immunity through MHC-II receptor checkpoint engagement

Justin M Balko¹, Douglas B Johnson¹, Paula Ericsson-Gonzalez¹, Mellissa J Nixon¹, Roberto Salgado², Violeta Sanchez¹, Daniel M Shreeder³, David L Rimm⁴, Ju Young Kim⁶, Jennifer Bordeaux⁶, Melinda E Sanders¹ and Randall S Davis⁷.
¹Vanderbilt University Medical Center; ²GZA and Jules Bordet Institute; ³University of Pennsylvania; ⁴Yale University; ⁵Peter MacCallum Cancer Center; ⁶Navigate BioPharma Services, Inc., a Novartis Company and ⁷University of Alabama.

Body: Background: We have previously shown that some breast cancers express major histocompatibility complex II (MHC-II), correlating with enhanced immune infiltration. In other tumor types, we have shown that MHC-II expression on tumor cells predicts clinical response to checkpoint inhibition. We sought to determine the direct effects of MHC-II on anti-tumor immunity and characterize mechanisms of immune escape in this breast cancer subset.

Methods: To determine the functional effects of MHC-II on tumor cells, we generated isogenic mouse breast tumor cells with enforced MHC-II expression and determined their ability to generate tumors in syngeneic mice, the impact on immunity, and their response to checkpoint inhibition. In a series of molecularly-characterized HER2+ (n=8) and triple-negative breast cancers (TNBC; n=103), we performed immunohistochemistry (IHC) and quantitative immunofluorescence (QIF) for Lag-3, PD-L1, CD4, CD8, FCRL6, and granzyme B.

Results: Following injection in syngeneic immunocompetent mice, MHC-II+ mouse breast tumors were more frequently rejected (p=0.04) and recruited greater numbers of CD4+ TILs. When MHC-II+ tumors escaped rejection, they expressed higher degrees of PD-1 and Lag-3 in the tumor and in the draining lymph node. Since Lag-3 is a checkpoint that specifically targets MHC-II, we hypothesized that MHC-II+ breast cancers escape anti-tumor immunity through suppressing MHC-II-mediated antigen presentation. Combinations of anti-Lag-3 and anti-Pd-1 antibodies inhibited growth of MHC-II+ tumors. These findings led us to also explore Fc receptor-like 6 (FCRL6), a previously reported MHC-II receptor expressed on NK and cytotoxic T cells. Residual MHC-II+ TNBC post-neoadjuvant chemotherapy (NAC) recruited greater numbers of CD4+ and CD8+ TILs (p=0.0001 and p=0.0002), suggesting enhanced immune recognition. However, MHC-II+ TNBCs also demonstrated a greater frequency of Lag-3+ and FCRL6+ TILs (p<0.001 and p=0.01, respectively) which frequently co-occurred (p=0.003). Thus, our data suggest that MHC-II expression in breast tumors supports recruitment of MHC-II-specific checkpoint-positive TILs. In line with this concept, QIF analysis demonstrated that the presence of Lag3+ and/or FCRL6+ TILs was strongly associated with suppression of T cell cytotoxicity as assessed by granzyme-B+ CD8+ T cells (p=0.0001 and p=0.002, respectively). Functional analyses of FCRL6 on human NK cell lines and peripheral blood mononuclear cells (PBMCs) demonstrated that like Lag3, FCRL6 is a checkpoint which engages MHC-II and suppresses cytotoxic NK and T cell activity.

Conclusions: These data suggest that MHC-II+ breast tumors are immunologically active and circumvent anti-tumor immunity by targeting MHC-II antigen presentation through recruitment of Lag-3+ and FCRL6+ TILs. We describe herein FCRL6 as a novel bona fide immune checkpoint which targets MHC-II, which may impact a variety of cancers. MHC-II expression status may be a useful biomarker for patient stratification on anti-PD-1/anti-Lag-3 combination, and eventually, anti-PD-1/anti-FCRL6 combinations in patients with breast cancer.
Title: Phase I-II trial of pembrolizumab and either doxorubicin (Dox) or an aromatase inhibitor (AI) for triple negative (TN) or hormone receptor positive (HR+) metastatic breast cancer (MBC)

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Body: Background: Metastatic TN and HR+ BC are incurable with current therapies. Preliminary data suggest modest activity with the PD-1 inhibitor pembrolizumab (pembro) in MBC. The addition of dox may increase expression of neoantigens enhancing PD-1 activity, while an AI effect may be enhanced by the PD-1 inhibitor in HR+ disease.

Methods: Primary endpoints were response and secondary endpoints included clinical benefit, safety, and progression-free survival. Cohort 1 includes patients (pts) without prior exposure to an anthracycline. Pts received escalating doses of dox at 50 and 60 mg/m2 to define the recommended phase 2 dose (RP2D) for a maximum of 6 cycles as well as pembro at 200 mg/every 3 weeks for up to 2 years. Cohort 2 includes 20 HR+ patients with treatment consisting of pembro 200 mg/every 3 weeks, and any AI to which a pt has not been exposed previously (exemestane preferred). PD-L1 expression was assessed by central laboratory-conducted immunohistochemistry. Peripheral blood samples before, during, and after treatment were collected and analyzed for cytokine production by Luminex multiplex assays and for immune cell markers by using flow cytometry. Biopsies were collected to assess immune profiles by using the Vectra automated quantitative pathology imaging system. CD8 T cells from the peripheral blood and FFPE slides were isolated before, during, and after treatment and T-cell receptor repertoires were compared to identify neoantigen-specific T cell clonotypes.

Results: Cohort 1 (TN MBC) accrued 3 pts without dose-limiting toxicities at 50mg/m2 with no grade 3 AEs, resulting in 1 partial response (PR), 1 unconfirmed PR, and 1 pt without progression for 7 cycles. At 60mg/m2, Cohort 2 is accruing and the RP2D will be reported. Cohort 2 (HR+ MBC) completed accrual of the planned 20 pts. The median age was 54, (range 43-78). There was 1 unconfirmed PR (still on after 3 cycles), 1 SD (progressed after 12 cycles), and 3 other patients are still on after 1-2 cycles. 1 pt came off for grade 3 liver enzyme (LFT) abnormalities in cycle 1, 14 pts progressed within 4 cycles. The median number of cycles is 2.5 (range 1-12). Grade 3 toxicities included 1 LFT abnormality, 1 grade 3 rash, and 1 grade 3 lymphocytopenia. Grade 2 toxicities were fatigue (N=5), rash (N=3), and 1 each of cough, headache, hot flashes, hypertension, insomnia, arthralgia and ankle edema. Plasma cytokines, blood and tumor immune profiles, and T cell clonotypes are under analysis.

Conclusions: The combination of dox or an AI with pembro is feasible. In the cohort of HR+ pts limited activity was observed and correlative analysis is underway to explore opportunities for patient selection. Responses were seen with the dox combination, and the RP2D will be reported on, along with additional follow-up. Early proliferation of peripheral blood T cell subsets may be a potential on-treatment biomarker to identify the MBC subset that will benefit from the additional of pembrolizumab and a full analysis of correlative studies is underway in the TN cohort to better guide effective and efficient cancer immunotherapy.
Title: A phase I trial of chemotherapy followed by infusions of activated T cells armed with anti-CD3 and anti-HER2 bispecific antibody for stage III, Her2+ or Her2− breast cancer

Patrick Dillon¹, Ritesh Rathore², Archana Thakur¹, Gerald Colvin², Nicola Kouttab² and Lawrence Lum¹. ¹University of Virginia, Charlottesville, VA and ²Roger Williams Medical Center, Providence, RI.

Body: Background: The balance in the immune system between immune surveillance and tolerance is known to be associated with the prognosis of breast cancer patients. The aim of this phase I study was to assess the safety of anti-CD3 x anti-HER2Bi bispecific antibody targeted (BAT) activated T cells (TC) in high risk breast cancer patients. The BAT T-cells exhibit anti-HER2 cytotoxicity, proliferate, and secrete immunokines upon tumor engagement.

Methods: High risk adjuvant breast cancer patients were recruited and completed standard adjuvant chemotherapy. BATs were produced by stimulating peripheral blood mononuclear cells (PBMC) obtained by leukapheresis; collected TC were then activated with anti-CD3 monoclonal antibody and expanded in IL-2 for 12-14 days. TC were armed with bispecific antibody and cryopreserved until used. Groups of 3 patients received 20, 40, 80 or 160 x 10⁹ BATs per infusion twice a week for four weeks. All patients were treated at Roger Williams Medical Center.

Results: Nine patients were accrued and all had N3 disease. Eight of 9 patients were ER positive; 2 of 9 were HER2 overexpressing. The median OS has not reached as five of nine patients are still alive. OS range from 14.3 to 154.7 months (as December 11, 2016). Five out of the five patients who are alive have no evidence of disease and 1 patient had a secondary primary that has been successfully treated and she has no evidence of disease. It was feasible to grow up to 160 x 10⁹ BATs and this dose level was tolerable without any cell-based dose limiting toxicities. BATs persisted in the blood for at least a week. BAT infusions induce cellular anti-tumor responses and cytokine responses.

Conclusion: Targeting HER2 positive and negative tumors induced cytotoxic anti-tumor responses, increases in Th1, cytokines and IL-12 serum levels. The prolonged survival in a high risk population suggests that BAT infusions provided a clinical benefit. These results are being confirmed in a phase II trial for metastatic breast cancer.
Title: Evaluating the anti-tumour efficacy of HAGE-derived vaccines in pre-clinical models

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Body: Background: The management of patients with TNBC continues to present a significant clinical challenge and the prognosis of patients remains poor due to the lack of targeting structures for existing therapies. We have found that the cancer testis antigen HAGE (DDX43, CT13) is expressed in 43% of patients with TNBC and that it is an independent predictor of a patient's response to chemotherapy. Moreover, patients with HAGE-positive residual disease after neoadjuvant therapy have an increased risk of recurrence and death (Abdel-Fatah et al., 2016). Therefore, patients with HAGE positive tumours might benefit from a HAGE-specific vaccine.

Aim: To determine the anti-tumour efficacy of peptide and DNA-based HAGE-derived vaccines.

Methods: HHDII/DR1 transgenic mice were vaccinated with a 30mer HAGE sequence either in the form of a peptide + adjuvants (CpG vs CAF09) or as a DNA vaccine, Immunobody®. Seven days after the last injection, splenocytes were harvested and peptide-specific immune response towards individual vaccine-derived shorter peptides were determined using an IFNγ ELISpot assay. The ability of splenocytes derived from immunised mice to recognise HAGE+ tumour cells after one week in vitro stimulation with HAGE-derived peptides was also determined. The anti-tumour efficacy of the vaccines was also tested against established HAGE+/HHDII+ tumours in pre-clinical models.

Results: Both peptide with adjuvants and the Immunobody® generated strong anti-HAGE immune responses, as demonstrated by the significant increase in the number of IFNγ secreting splenocytes. Moreover, splenocytes from vaccinated mice stimulated in vitro could recognise and specifically respond to HAGE+ tumour cells. This response was both HAGE and CD8+ T cell-specific. Vaccination with HAGE 30mer vaccine significantly slowed the growth of HAGE+/HHDII+ tumours.

Conclusion: Cancer testis antigens such as HAGE / DDX43 are ideal candidates for anti-cancer therapies due to their restricted expression in normal tissue and over-expression in cancer tissues. However, many of these antigens suffer from poor immunogenicity, and therefore require strong adjuvants and/or appropriate delivery systems. The adjuvants/delivery system tested in this study show promising results. Future work will investigate combination approaches using checkpoint inhibitors. Overall, demonstrating potential value of HAGE-derived vaccines for the treatment HAGE positive cancers will underpin the development of a platform for a phase I clinical trial in the relevant patient group(s) upon demonstrating potential value of HAGE-derived vaccines for the treatment HAGE positive cancers.
Title: A phase 1b study of abemaciclib plus pembrolizumab for patients with hormone receptor-positive (HR+), human epidermal growth factor receptor 2-negative (HER2-) metastatic breast cancer (MBC)

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Body: Background: Abemaciclib is a selective and potent small molecule inhibitor of CDK4 and 6, with evidence of single-agent antitumor activity, and a safety profile that enables dosing on a continuous schedule. Abemaciclib demonstrated anti-tumor activity as a single agent with a 19.7% objective response rate for women with previously treated HR+, HER2- MBC ¹. In the phase 1b study JPBH, abemaciclib demonstrated a tolerable safety profile when combined with endocrine or HER2 targeted agents for MBC ². Abemaciclib given BID in combination with pembrolizumab also demonstrated a tolerable safety profile in phase 1b study of stage IV NSCLC ³.

Methods: JPCE is a multicenter, nonrandomized, open-label, phase 1b study of abemaciclib plus pembrolizumab for patients with HR+, HER2- MBC or NSCLC (ClinicalTrials.gov NCT02779751). The study has 3 disease-specific cohorts, each with approximately 25 patients (N=75); the HR+, HER2- MBC cohort (part C) will be presented here. The primary objective was to characterize safety of the abemaciclib and pembrolizumab combination. Secondary objectives included efficacy endpoints (objective response rate, disease control rate, duration of response, progression-free survival, and overall survival), pharmacokinetics of abemaciclib plus pembrolizumab, and changes in patient-reported pain and disease-related symptoms. Patients received 150 mg of abemaciclib orally Q12H plus pembrolizumab 200 mg as a 30-minute IV infusion on Day 1 every 21 days. Eligible patients included women with confirmed HR+, HER2- MBC who have previously received at least 1 but no more than 2 prior chemotherapy regimens for MBC; are able to provide tumor tissue at baseline and at cycle 3, day 1; have measurable disease (RECIST v.1.1), adequate organ function, ECOG PS ≤1, are able to swallow oral medications; and have not received treatment with CDK4 & 6 or PD-1/ PD-L1 inhibitors.

Results: At the time of abstract submission, study JPCE part C cohort (HR+, HER2- MBC) was fully enrolled at 25 patients. Data to be presented include patient demographics, baseline disease characteristics, adverse events by frequency and by grade, and preliminary efficacy of the abemaciclib plus pembrolizumab combination in HR+, HER2- MBC.

References:
1. Dickler et al, Clin Cancer Res. 2017
2. Goetz et al. poster presented at SABCS, 2015
Title: Bromodomain inhibitors for the treatment of invasive lobular carcinoma

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Body: Invasive lobular carcinoma (ILC) is the second most common type of breast cancer after invasive ductal carcinoma (IDC), accounting for approximately 10-15% of all breast tumors. ILC is characterized by inactivation of E-Cadherin and neoplastic cells that invade the stroma in a "single-file" pattern. Women with ILC are usually older, have used hormone replacement therapy and are more likely to have hormone receptor–positive disease. ILCs have similar survival to IDCs at both five and 10 years, but despite this, the clinical course is distinct: ILCs are three times more likely to metastasize to the peritoneum, gastrointestinal tract, and ovaries and are more frequently bilateral. Therefore, tailored therapeutic options for this distinct, hard-to-treat subtype of breast cancer are required.

As part of the RATHER FP7 HEALTH consortium (www.ratherproject.com), we carried out RNA-Seq analysis of 61 primary ILC samples and identified that high expression of the BET family protein Brd3 (uniquely among BRD family members) was associated with poor recurrence free survival (p=0.03, HR 8.63, CI 1.22-60.85). This observation was further validated in the independent METABRIC cohort (n=99), where again, high Brd3 expression (and not other BRD members) was associated with poor recurrence-free survival (p<0.01, HR=3.16, CI 1.24-8.03). Using a two ILC cell lines (SUM44PE and MDA-MB134VI) we found that ILC cells were relatively resistant to the anti-estrogen therapies tamoxifen and fulvestrant compared to those derived from IDC. Next, we tested whether the ILC cell lines were sensitive to BET protein inhibition using the pan-BET family inhibitor JQ1. Interestingly, while JQ1 inhibited cell growth in both ILC cell lines tested, apoptosis was only induced in SUM44PE cells, while MDA-MB134VI cells exhibited G1 arrest. Dynamic BH3 profiling was used to dissect the underlying anti-apoptotic dependencies in each ILC cell type and showed that in the JQ1-resistant MDA-MB134VI cells, survival was predominantly Bcl2-dependent. Combination of JQ1 and the Bcl2-inhibitor venetoclax (ABT-199) synergistically killed MDA-MB134VI cells compared to treatment with JQ1 alone, while combination with the Bcl2/Bcl-Xl/Bcl-W inhibitor navitoclax (ABT-263) added further synergy.

With a number of BET inhibitors now entering clinical trials, the data described here suggest that BET inhibition is a rational therapeutic option for some ILC cases, and for those that do not respond, combination with venetoclax may be a suitable therapeutic strategy. In our cell line models, baseline Bcl-2 expression was sufficient to predict induction of apoptosis in response to JQ1 and could be used to guide therapeutic choice. These results should now be investigated in vivo before a prospective clinical trial.

This material is based upon works supported by the Irish Cancer Society Collaborative Cancer Research Centre BREAST-PREDICT Grant CCRC13GAL" and the SFi CDA Award 15/CDA/3438
Global knockdown of cellular kinases identifies MPS1 as a novel modulator of endocrine and palbociclib resistance highlighting a new role for MPS1 inhibitors

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Body: Background Estrogen-receptor positive (ER+) breast cancer (BC) accounts for over 75% of diagnosed cases. Despite treatment, a large proportion relapse with de novo or acquired endocrine resistant disease, making it one of the greatest challenges for BC research. Using a kinome siRNA library screen, we identified MPS1 that is required for recruitment of the spindle assembly checkpoint complex, as strongly associated with resistance to endocrine therapy and palbociclib. Until now, the target population for MPS1 inhibitors has focused on triple negative BC. Our unexpected finding shows, potential efficacy of MPS1 inhibitors in ER+ BC resistant to endocrine therapies and palbociclib, a prominent contemporary first-line combination for advanced disease.

Methods ER+ BC cell lines (MCF7, SUM44, ZR75.1, HCC1428 and T47D) adapted to estrogen independent growth (LTED) and sequential resistance to palbociclib (991R) were subjected to a siRNA screen targeting 709 kinases. Z-scores were used to identify the most robust candidates. Cell viability upon MPS1 inhibition with CCT289346 (MPS1i) was assessed 2D and 3D. The class effect was confirmed with other compounds targeting MPS1. Impact of MPS1i on ER co-localisation and ER-transactivation was assessed using confocal microscopy and reporter assays, respectively. Effect of MPS1i on chromosomal alignment and time spent in mitosis was established by time lapse and confocal microscopy. BrdU incorporation and cell cycle were assessed by FACS. PARP cleavage was used to measure apoptosis. Global gene expression analysis of MPS1 was carried out in two independent neoadjuvant studies of aromatase inhibitor (AI) treated patients.

Results Kinome knockdown identified targets associated with the G2/M checkpoint as strongly implicated in the LTED phenotype. In particular, MPS1 was the top common hit in LTED and 991R cell lines. Increase in MPS1 was evident in MCF7-LTED at both the transcript and protein level. Notably, the MPS1 inhibitor CCT289346 caused a significant reduction in viability of the majority of LTED and 991R cell lines tested.

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<th>Cell Line</th>
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<td>MCF7</td>
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<td>LTEDESR1wt</td>
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<td>T47D</td>
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Upon inhibition of MPS1, cells demonstrated shorter time in mitosis, aberration of cell cycle and amplified mitotic errors, resulting in increased apoptosis.
To evaluate the clinical relevance of \textit{MPS1} in ER+ BC treated with endocrine therapy, we interrogated publicly available datasets from patients treated with neoadjuvant AI therapy. In the anastrozole cohort, on-treatment gene expression of \textit{MPS1} (p<0.0001) was significantly associated with poor response to anastrozole based on a 2-week residual Ki67 score <10%. In the letrozole cohort, increased on-treatment expression of \textit{MPS1} (p=0.0118) was associated with poor response based of tumor shrinkage ≥50%.

\textbf{Conclusion} This novel finding shows MPS1 inhibitors are capable of inducing mitotic aberrations and apoptosis in ER+ BC models resistant to endocrine therapy and palbociclib providing a new therapeutic strategy.
Activity of CT7001 an orally bio-available cyclin-dependent kinase 7 selective inhibitor in models of triple negative breast cancer

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Body: Triple-negative breast cancer (TNBC) is a highly aggressive and heterogeneous subtype of breast cancer that commonly exhibit poor prognosis and high relapse rates at early stages after conventional neoadjuvant chemotherapy. CDK7 inhibition has emerged as an ‘Achilles heel’ in TNBC via blocking transcriptional addiction to a defined cluster of genes (Wang et al 2015). CDK7 acts as a CDK-activating kinase controlling proliferation and as a transcriptional kinase phosphorylating RNA Polymerase II. Eukaryotic RNA polymerase II (Pol II) is a 12-subunit DNA-dependent RNA polymerase that is responsible for transcribing nuclear genes encoding messenger RNAs and several small nuclear RNAs (R Young 1991)

We have demonstrated that established cell-lines and patient derived tumour tissue (PDTT, explants established as models at low passage numbers that have not been grown in plastic or propagated as cell cultures) of TNBC are sensitive to a potent, selective and orally bioavailable CDK7 inhibitor CT7001 (ICEC0942). CT7001 produces a concentration-dependent inhibition of growth with GI50s <1 micromolar across all TNBC cells tested to-date. The inhibition of proliferation was associated with an inhibition of c-MYC, Mcl-1 and phospho-Pol II as determined by Western Blot analysis. This demonstrates that CT7001 effectively controls transcriptional regulation and anti-apoptotic mechanisms in a diverse group of TNBC cellular models.

CT7001 was also evaluated in an in vivo orthotopic-PDX model of TNBC in nu/nu mice. Establishing PDX-xenograft tumour models from PDTT at low passage is believed to conserve original tumour characteristics such as heterogeneous histology, clinical biomolecular signature, malignant phenotypes and genotypes. Therefore, patient-derived tumour grafts are believed to offer relevant predictive insights into clinical outcomes when evaluating the efficacy of novel cancer therapies. Orally administered CT7001 monotherapy produced strong and sustained regression of the tumour that persisted during the dosing schedule and strong suppression was still maintained upon cessation of treatment. At doses that produced regression CT7001 was well tolerated with little effect on body weight loss (<10%)

CT7001 is a potent, selective and orally bioavailable inhibitor of CDK7 that shows promise as a potential new treatment for TNBC.

References
Radiosensitization of androgen receptor (AR)-positive triple-negative breast cancer (TNBC) cells using seviteronel (INO-464), a selective CYP17 lyase and AR inhibitor

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Body: Background: Increased rates of locoregional recurrence (LR) have been observed in TNBC despite chemotherapy and radiation (RT). A novel radiosensitizer screen nominated the AR as a promising target for treatment of radioresistant breast cancer, including TNBC. We assessed the activity of seviteronel (Sevi), a selective CYP17 lyase and AR inhibitor in Phase 2 clinical development for advanced breast and prostate cancer, as a potential radiosensitizer in AR+ TNBC model.

Methods: Clonogenic survival assays were used to determine the intrinsic RT sensitivity of 21 breast cancer cell (BCC) lines. IC50 values were determined for 130 clinically available compounds and correlation coefficients were calculated using IC50 values and SF-2Gy. Gene expression was measured using RNA Seq or qRT-PCR and protein expression was measured using RPPA arrays. AR function was assessed using functional inhibition with Sevi in MDA-MB-453, ACC-422, ACC-460, SUM-185 (all four AR+ TNBC), MDA-MB-231 (AR- TNBC), and T47D (AR- ER+) BCC lines. Double-stranded DNA (dsDNA) break repair was assessed with γH2AX foci counting. In vivo tumor growth was measured with varying control and treatment groups (16-20 tumors/group). Kaplan-Meier analysis was performed to estimate local control. A Cox proportional hazards model and multi-variate analysis (MVA) were used to determine variables associated with LRF survival.

Results: Our novel radiosensitizer screen identified the activity of anti-androgen therapy as a potentially effective strategy for radiosensitization in RT-resistant BCC lines (R² =0.46, p-value < 0.01) (Speers et al, J Clin Oncol 35, 2017 (suppl; abstr e12102). Heterogeneity in AR expression was identified in human BCC lines and TNBC samples from patients (N=2098). There was a strong correlation between AR RNA expression and protein expression across all BC intrinsic subtypes. AR inhibition using Sevi induced radiation sensitivity in vitro with an enhancement ratio (ER) of 1.24-1.69 in four different AR+ TNBC lines. No such radiosensitization was seen in AR(-) TNBC or ER+, AR(-) BCC lines. Radiosensitization was at least partially dependent on impaired dsDNA break repair with significant delays in dsDNA break repair at 16 and 24 hours in all AR+ TNBC lines examined (p-value < 0.01). AR inhibition with Sevi significantly radiosensitized AR+ TNBC xenografts in mouse models and markedly delayed tumor-volume tripling time (TTT) and tumor growth (MDA-MB-453: median TTT 16.1 days for RT alone vs. not reached after 45 days for Sevi+RT, p-value <0.001). Similar delays were seen in tumor growth, weight, and tumor doubling. Clinically, TNBC patients whose tumors had higher than median expression of AR had higher rates of LR after RT (HR for LR ~3, p-value <0.01, 2 independent datasets). In MVA, high AR expression was the variable most significantly associated with worse LR survival after RT in TNBC patients, outperforming all other variables (HR of 3.42; p-value < 0.01).

Conclusions: Our results implicate the AR as a mediator of radioresistance in breast cancer and support the rationale for developing Sevi as a novel radiosensitizing agent in AR+ TNBC.
Blocking ER coregulator signaling enhances CDK4/6 inhibitor palbociclib therapy in ER-positive advanced breast cancer

Body: BACKGROUND: Recently, CDK4/6 inhibitors in combination with endocrine therapy (AE/AI/SERDs) is approved for the treatment of ER+ advanced breast cancer (BCa). However, not all patients benefit from CDK4/6 inhibitors therapy. Emerging studies indicate many therapy-resistant tumors retain ER signaling, via interaction with critical oncogenic coregulator proteins. Considering complex signaling interplay of ER and CDK4/6 axis, combination therapy of CDK inhibitor with other potent ER-targeted agents that block ER coregulatory signaling may extend the efficacy and may prevent the development of resistance to the CDK4/6 inhibitors. We recently developed a small organic molecule, ER coregulator binding modulator ERX-11 (EtiraRx-11). The objective of this study is to test the utility of novel combination therapy of ERX-11 with CDK4/6 inhibitor palbociclib in treating therapy resistant advanced BCa.

METHODS: We have utilized multiple therapy sensitive and therapy-resistant BCa models with various genetic backgrounds. We tested efficacy using both acquired resistance and engineered models that express ER mutations or oncogenes. Efficacy of combination therapy was tested using established in vitro assays including, MTT, colony formation, apoptosis, and cell cycle progression. Mechanistic studies were conducted using reporter gene assays, gene expression, RNA-seq analysis and signaling alterations. Patient-derived BCa explant and Xenograft studies were used to determine the in vivo efficacy of the combination therapy.

RESULTS: ERX-11 effectively blocked ER-mediated and ER-coregulator mediated oncogenic signaling and has potent anti-proliferative activity against both endocrine therapy-sensitive and therapy-resistant BCa cells. Mechanistic studies using IP-Mass spectrometry showed that ERX-11 blocks the interaction between a subset of coregulators with ER in resistant BCa models. ERX-11 exhibited potent anti-proliferative activity against therapy-sensitive and therapy-resistant ER-driven BCa cells in vitro, in xenograft models in vivo and in patient-derived breast tumor explants ex vivo. Co-treatment of ERX-11 with palbociclib synergistically reduced cell viability and induced apoptosis of therapy sensitive and resistant BCa model cells. Importantly, combination therapy of ERX-11 and the palbociclib synergistically reduced the growth and induced apoptosis of tamoxifen and letrozole resistant xenograft tumors compared to either drug alone. RNA-seq studies revealed that combinational treatment with ERX-11 and palbociclib uniquely activated p53 and unfolded response mediated apoptotic pathways and suppressed E2F and Myc target genes. Biochemical studies confirmed combination therapy significantly altered E2F1 and ER signaling pathways and promoted apoptosis.

CONCLUSIONS: Our data support a critical role of blocking ER coregulator signaling in treating therapy resistance in advanced ER+ BCa. Combinational treatment with ERX-11 and palbociclib may overcome/delay endocrine therapy resistance.
Title: Breast cancer initiating cells express functional ROR1, which can be targeted by cirmtuzumab to potentially mitigate the risk of relapse after therapy

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Body: Although initially responsive to chemotherapy, patients with advanced breast cancer often relapse, generally with incurable metastatic disease. This may be due to a subpopulation of tumor cells, called cancer-initiating cells, or cancer stem cells (CSCs), which are relatively resistant to chemotherapy and have self-renewing and tumor-initiating capacities. Prior studies in our laboratory found that CSCs may express ROR1, an onco-embryonic, tyrosine-kinase-like orphan receptor, which we found could bind Wnt5a to activate non-canonical Wnt-signaling (Proc Nat Acad Sci USA 111:17266, 2014). Interrogation of the transcriptomes of breast-cancer cells obtained from patients before and after paclitaxel therapy revealed that chemotherapy treatment enhanced cancer-cell expression of ROR1, along with genes induced by activation of Rho-GTPases (e.g. RhoA, cdc42, and Rac1). We found that primary breast-cancer patient-derived xenografts with high-level expression of ROR1 were enriched for cells that had activated Rho-GTPases and stem-cell-like gene-expression signatures. Furthermore, we found that treatment of breast cancer cell lines with Wnt5a induced ROR1-dependent activation of Rho-GTPases and AKT and induced high-level protein expression of BMI1, also known as polycomb group RING finger protein 4 (PCGF4) or RING finger protein 51 (RNF51); Wnt5a also enhanced the capacity of breast cancer cell lines to form spheroids. All these effects could be inhibited by cirmtuzumab, a humanized high-affinity anti-ROR1 mAb, which can block Wnt5a signaling. We find that ROR1-positive breast cancer cells have a greater capacity to form spheroids or engraft immune-deficient mice than did ROR1-negative cancer cells isolated from the same PDX tumor. Treatment of immune-deficient mice bearing breast-cancer PDX with paclitaxel reduced tumor volumes but enhanced expression of ROR1 and other CSC markers, such as aldehyde dehydrogenase 1 (ALDH1). Moreover, the breast cancer cells surviving such paclitaxel treatment had increased activation of Rho-GTPases and AKT, and increased expression of BMI1, relative to that of breast cancer cells obtained from the same primary tumor prior to therapy. On the other hand, treatment of such mice with cirmtuzumab also reduced breast cancer PDX tumor volumes, but the remaining cells had reduced expression of ROR1 and CSC markers and had impaired capacity to re-engage immune-deficient mice. Finally, therapy with cirmtuzumab and paclitaxel was more effective in eradicating breast-cancer PDX than treatment with either agent alone. Collectively, these findings support use of cirmtuzumab in combination with conventional anti-cancer drugs to improve the outcome of patients with advanced breast cancer.
Title: *BCL2L1* (BCL-XL) expression and MYC super-enhancer positivity predict sensitivity to the covalent CDK7 inhibitor SY-1365 in triple negative breast cancer (TNBC) cell lines

Nisha Rajagopal¹, Graeme Hodgson¹, Shanhu Hu¹, Michael McKeown¹, Alex Bush¹, Christian Fritz¹, David Orlando¹, Eric Olson¹ and Emmanuelle di Tomaso¹. ¹Syros Pharmaceuticals, Cambridge, MA.

Body: Effective therapies for TNBC remain elusive. As such, TNBCs are associated with a high risk of relapse and short progression free- and overall-survival. Recent studies showed that TNBC cells are highly dependent on the transcriptional regulator CDK7, and suggest that the mitochondrial apoptosis pathway is important in mediating cell survival in CDK7-dependent cells. Further, TNBC has been shown to have a distinct epigenetic and transcriptional program, with super-enhancers (SE) mediating the expression of key oncogenic drivers such as *MYC*. SY-1365, a covalent and selective inhibitor of CDK7, was developed to exploit dysregulated programs thought to drive SE-mediated transcriptional-dependencies in TNBC and other cancers. To identify potential biomarkers predictive of sensitivity to SY-1365, we evaluated SY-1365 inhibitory activity in a large panel of human tumor cell lines, including TNBC lines, and correlated sensitivity with RNA expression and epigenetic profiles.

SY-1365 dose-response curves were measured using the ATP-lite assay in a panel of 406 human tumor cell lines, including 19 TNBC cell lines. Clustering of growth-rate adjusted dose response curves of cell-lines treated with SY-1365 allowed the classification of cell-lines into low and high response groups. An unbiased genome wide approach was used to compare response classification to RNA expression data across all cell lines to identify gene expression markers predictive of sensitivity to SY-1365. Furthermore, a hypothesis driven approach was followed to interrogate whether the *MYC* SE predicted sensitivity to SY-1365.

Twenty-five genes were differentially expressed between SY-1365-sensitive and -insensitive tumor lines (FDR<0.05). Lower expression of *BCL2L1*, which encodes the mitochondrial apoptosis regulator BCL-XL, was identified as the most predictive expression biomarker of sensitivity across all profiled cell lines, strongly separating the two classes of sensitivity (Accuracy=70%, FDR<0.005). Further, this predictive power of lower *BCL2L1* expression was maintained in an analysis restricted to the subset of TNBC cell-lines (Accuracy=73%).

Expanding beyond expression analysis, we also found that the strength of the *MYC* SE (as defined by H3K27Ac) was predictive of response to SY-1365 in TNBC (Accuracy=86%, FDR<0.05).

In this study, we show for the first time that SY-1365 induced differential responses across a large panel of human tumor cell lines derived from multiple indications. We also show that in this panel of cell lines the response could be predicted in an “indication agnostic” manner by the level of expression of *BCL2L1*. Finally, in line with prior reports, in TNBC cell lines, MYC SE was significantly associated with sensitivity to SY-1365. These observations have generated strong hypotheses for selection strategies aimed at identifying patients with tumors particularly sensitive to CDK7 inhibition with SY-1365, and warrant further investigation with respect to predictive biomarkers of response in patients. SY-1365 is currently being assessed in a phase 1 trial in adult patients with advanced solid tumors, including a planned expansion cohort enriching for patients with TNBC (NCT03134638).
Title: Efficacy of estrogen receptor β agonists in the prevention of breast cancer progression to therapy resistance

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Body: Estrogen plays an important role in the initiation and progression of breast cancer (BCa). Approximately, 70% of breast tumors are estrogen receptor (ER) positive at the time of presentation. Endocrine therapy using aromatase inhibitors (AI), or anti-estrogen (AE) molecules are widely used for treating ER+ve BCa. However, their efficacy is limited by intrinsic and acquired therapy resistance and most patients develop resistance to these drugs. The transcriptional effects of estrogen are mediated by two ERs (ERα and ERβ) and both are expressed in normal breast tissue. Unlike ERα, ERβ functions as tumor suppressor. However, role of ERβ specific agonists in the prevention of BCa progression remains elusive. In this study, we investigated the effectiveness of two ERβ agonists (S-Equol and LY500307) in the prevention of BCa progression using endocrine therapy sensitive (MCF7-aro) and letrozole resistant (MCF7-aro-LTLT) cells. Our results demonstrated that treatment with ERβ agonists inhibit short-and long-term growth of both endocrine therapy sensitive and resistant BCa cells. In addition, ERβ agonists treatment inhibited invasion and migration of both MCF7-aro and MCF7-aro-LTLT cells. Importantly, cell cycle analysis revealed that ERβ agonists induced cell cycle arrest. Our gene microarray analysis demonstrated that both ERβ agonists significantly modulated genes involved in the cell cycle progression, DNA replication and cell death pathways. Further, gene enrichment analysis of differentially expressed genes revealed that genes involved in the cell cycle checkpoints emerged as significant pathway modulated by ERβ agonists treatment in MCF7-aro cells. Interestingly, in letrozole-resistant MCF7-aro cells, DNA replication was significantly affected by ERβ agonists treatment. Pathway analysis also identified enrichment for chemokine signaling pathways. We confirmed pathway analysis by qRT-PCR and western blot analysis. Accordingly, treatment of in vivo syngeneic xenografts with ERβ agonists significantly inhibited BCa progression. Collectively, these results from this study suggest that ERβ agonists have potential to prevent the progression of BCa progression.
Title: ICOSL anti-HER2 V-mAbs: Localizing engineered ICOSL costimulatory agonists to HER2+ tumors through trastuzumab

Erika Rickel1, Lawrence Evans1, Ryan Swanson1, Steve S Levin1, Mark Rixon1, Martin Wolfson1, Janhavi Bhandari1, Sean MacNeil1, Joe Hoover1, Michael Kornacker1, Irene Capuano1 and Stanford L Peng1. 1Alpine Immune Sciences, Seattle, WA.

Body: Background: The presence of tumor infiltrating lymphocytes (TILs) has been associated with improved prognosis in HER2+ breast cancer patients. Antigen specific TCR and costimulatory receptor signaling drive increases in TIL number, effector function, and tumor cytotoxicity. Improving the number and effector phenotype of tumor localized TILs has curative potential by enhancing the adaptive and memory immune response. Targeting HER2 with the monoclonal anti-HER2 antibody trastuzumab has improved survival in HER2+ breast cancer patients and is known to increase peripheral type I immunity, which may be reflected by increased TILs.

The Immunoglobulin Superfamily (IgSF) includes a large, diverse family of immunotherapy targets expressed on immune cells and tumors. Transmembrane IgSF receptors, CD28 and inducible T cell co-stimulator (ICOS), related costimulatory molecules expressed on T cells, interact with CD80/CD86 and ICOS ligand (ICOSL), respectively, and play critical roles in T cell activation and adaptive immunity. The Alpine Immune Science's vIgD™ platform uses directed evolution to derive novel, therapeutically-applicable IgSF extracellular domains with tailored specificity and affinity. The vIgD platform has generated human ICOSL vIgDs capable of binding both ICOS and CD28, activating both pathways. To promote anti-tumor activity of TILs in HER2+ tumors, we developed trastuzumab-ICOSL “V-mAbs” consisting of trastuzumab fused to activating ICOSL vIgDs. These V-mAbs are designed to localize to HER2+ tumors and activate antigen-specific, resident T-cells through costimulatory receptor agonism.

Methods: V-mAbs were generated by fusing ICOSL vIgDs to either the N- or C-termini of the heavy and/or light chains of trastuzumab. V-mAb binding to CD28, ICOS or HER2 was measured by flow cytometric analysis of transfected cells or ForteBio analysis. V-mAb costimulatory activity was confirmed by immobilization in the presence of anti-CD3 in a primary human T cell assay. Finally, V-mAbs were co-cultured with HER2+ target cells and human T cells; T-cell activity was measured by proliferation, cytokine production, and target lysis.

Results: V-mAbs were successfully produced and bound to CD28, ICOS and HER2. In a plate bound costimulation assay, the V-mAbs increased the amount of IFN-gamma produced by T-cells stimulated with anti-CD3. When incubated with HER2+ target cells, V-mAbs promoted T-cell proliferation, cytokine secretion, and target cell lysis. Data from in vivo studies, to determine the impact of trastuzumab V-mAbs on HER2+ cancers, will be presented when available.

Conclusions: Trastuzumab-ICOSL V-mAbs are novel ICOS- and CD28-activating immunotherapies for HER2-positive tumors, promoting T-cell proliferation, cytokine secretion, and target cell lysis in a HER2 dependent fashion. The V-mAb platform has broad potential to enable tumor-localized immune modulation via the diverse array of IgSF members. Preclinical development of trastuzumab-ICOSL clinical therapeutics is in progress.
Title: A phase Ib study of oral administration of lucitanib in combination with fulvestrant in patients with HR+ metastatic breast cancer (mBC)

Mario Campone¹, Thomas Bachelot², Frederique Renault-Llorca³, Athanasios Pallis⁴, Valerie Agrapart⁴, Marie-Jeanne Pierrat⁴, Camille Poirot⁴, Gauthier Paux⁴, Frederic Dubois⁴, Laura Xuereb⁴, Renata Robert⁵ and Fabrice Andre⁵. ¹Institut de Cancérologie de l'Ouest – Centre René Gauducheau, Saint-Herblain, France; ²Centre Léon Bérard Centre de Lutte Contre le Cancer (CLCC) de Lyon, Lyon, France; ³Centre Jean Perrin, Clermont-Ferrand, France; ⁴Institut de Recherches Internationales Servier, Suresnes, France and ⁵Institut Gustave Roussy, Villejuif, France.

Body: FGFR1 amplification could mediate resistance to endocrine therapy and FGFR1 inhibition reverses this resistance. This phase Ib seeks to evaluate whether the combination of lucitanib, a potent FGFR/VEGFR/PDFGR inhibitor, in combination with fulvestrant, an endocrine agent, reverses resistance to fulvestrant.

Eligible patients for this study were postmenopausal with ER+/HER2- mBC and have relapsed during or after treatment with fulvestrant. There were 2 parts in the study: a dose allocation to assess the tolerability of the combination in terms of DLTs and MTD using a modified Continual Reassessment Method (mCRM) [part I] and a dose expansion, with patients assigned to 2 different cohorts based on FGFR amplification, to further evaluate the tolerability of the combination and to identify the recommended phase II dose (RP2D) [part II]. Surrogate target hitting biomarkers were also dosed at baseline and on-treatment. The sponsor decided to halt the clinical development in mBC indication and the study was prematurely terminated after 18 patients (15 in part I and 3 in part II). The presentation will focus on these 18 patients.

Patients had ECOG PS 0 or 1 and median number of previous treatments in metastatic setting was 3. Two doses of lucitanib (10mg daily n=9 and 12.5mg daily n=6) in combination with 500 mg/month of fulvestrant were tested in part I. At the 10mg dose level, one patient experienced a DLT (grade 3 hypertension). Based on global lucitanib development program data, it was decided to start Part II with lucitanib 10mg daily. The most common related grade ≥3 toxicities occurring in more than 10% of patients were hypertension (78%) and asthenia (22%). All patients required at least one dose interruption mainly for toxicities, while 13 patients (72%) required at least a dose reduction for toxicities. Thirteen patients (72%) withdrew from the study for disease progression, 3 (17%) withdrew from the study for adverse events (at 10mg) and 2 (11%) for non-medical reasons. Three patients achieved a confirmed partial response (as per RECIST v1.1), one at 10mg and two at 12.5mg. About 55% of the patients experienced clinical benefit with a median duration of the benefit of 39.6 weeks and a maximum duration of the benefit of 79.1 weeks for 1 patient (PR at Cycle 4). Biomarker modulations were consistent with lucitanib mode of action; targeting VEGFRs (significant increase of VEGFA, IL8, PIGF) and FGFR1 (significant increase of FGF23).

The combination is feasible but requires close patient monitoring and intensive management of adverse events. Those are in line with the anti-angiogenic activity of lucitanib.

<table>
<thead>
<tr>
<th>Objective Response Rate (ORR)</th>
<th>10mg (N=12)</th>
<th>12.5mg (N=6)</th>
<th>All (N=18)</th>
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</thead>
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<tr>
<td>n(%)</td>
<td>1 (8.3)</td>
<td>2 (33.3)</td>
<td>3 (16.7)</td>
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<tr>
<td>95% CI</td>
<td>[1.5;35.4]</td>
<td>[9.7;70.0]</td>
<td>[5.8;39.2]</td>
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<tr>
<td>Clinical Benefit Rate (CBR)</td>
<td>4 (33.3)</td>
<td>6 (100.0)</td>
<td>10 (55.6)</td>
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<tr>
<td>n(%)</td>
<td>[13.8;61.0]</td>
<td>[61.0;100.0]</td>
<td>[33.7;75.4]</td>
</tr>
<tr>
<td>95% CI</td>
<td>[27.9 ; 32.7]</td>
<td>[29.1 ; 79.1]</td>
<td>[27.9 ; 79.1]</td>
</tr>
</tbody>
</table>

1: CR or PR 2: CR or PR or stabilization (SD or NonCR/NonPD) >24 weeks or at end of cycle 6 3: 95% Wilson method of Confidence interval of the estimate
Title: mTORC1 is responsible for metastasis of ER+ breast cancer downstream of RON tyrosine kinase

Najme Faham1 and Alana welm1. 1Huntsman Cancer Institute, Salt Lake City, UT.

Body: Metastatic breast cancer is one of the major challenges of tumor biology, remaining the underlying cause of death in the vast majority of breast cancer patients. Despite advances in treatment strategies, metastatic breast cancer is still considered incurable and mostly resistant to the current treatment options. There is, therefore, a clear need for development of novel targeted therapeutic agents, which must be built on a foundation of a deeper understanding of the molecular mechanisms responsible for development of metastasis. It is well accepted that aberrant expression of the RON receptor tyrosine kinase in breast cancer contributes to development of metastasis, and is associated with poor patient prognosis. Elevated expression of RON has been reported in about 50% of primary breast cancer samples. However, in later metastatic stage, RON is overexpressed in 100% of breast cancer samples, pointing toward its important role in inducing metastasis.

RON is known to activate multiple signaling pathways, including MAPK, PI3K, Src, phospholipase C-γ and β-catenin. Although some of these pathways have been linked to specific biological outcomes in vitro, contributions of each signal transduction pathway to cancer progression and metastasis are still poorly defined. To address this critical question, we used a combination of inhibitory and mutational strategies. We engineered T47D cells to conditionally overexpress RON upon addition of doxycycline, enabling us to achieve titratable levels of RON. For inhibition of RON signaling, we used a highly selective RON inhibitor, ASLAN002. To obtain a broader knowledge about signaling networks downstream of RON, we used a high-throughput functional proteomics assay, Reverse Phase Protein Array (RPPA). RPPA analysis showed robust phosphorylation of ribosomal protein S6 (rpS6) upon RON activation, which could be reversed by RON inhibitor. We were then able to dissect the components of the pathway responsible for phosphorylation of rpS6 using inhibitors against different potential kinases. These analyses revealed that mTORC1 is the main kinase responsible for profound phosphorylation of rpS6 downstream of RON, which itself is fed dominantly by PI3K rather than MAPK. To validate the PI3K/mTORC1/p70S6K axis as the main feeder of rpS6, we took advantage of a mutational approach to modulate RON signaling to specific pathways. Biochemical analysis of various mutants in T47D cells showed that they signal differentially, with mutant A strongly signaling through PI3K/mTORC1. To assess the importance of PI3K/mTORC1 in RON-mediated metastasis, we conducted in vivo studies. Our results indicated that mutant A is the highest metastatic mutant, inducing metastasis in 100% of the mice studied. To examine whether mTORC1 is critical for induction of metastasis downstream of RON, we treated mice with mTORC1 inhibitor, everolimus, and monitored metastasis. Our data revealed that mTORC1 inhibition could dramatically shrink metastatic lesions, before resistance occurs.

We report a dissected analysis of the RON signaling pathway responsible for induction of metastasis in ER+ breast cancer. Our data strongly indicate that RON dominantly signals through PI3K/mTORC1/p70S6K/rpS6, and that this pathway is important in inducing metastasis.
**Title:** Safety and efficacy of neoadjuvant metformin with trastuzumab and chemotherapy in women with HER2-positive early breast cancer: A randomized, open-label, multicenter, phase 2 trial

Sonia Pernas¹, Joan Dorca², Isabel Álvarez-López², Susana Martínez³, Cristina Saura⁴, Norberto Batista López⁵, César A Rodríguez-Sánchez⁶, Kepa Amillano⁷, Severina Domínguez-Fernández⁸, María Luque Cabal⁹, Idoia Morilla¹, Gemma Viñas¹, Javier Cortés⁴, Elisabet Cuyàs¹⁰, Sara Verdura¹⁰, Salvador Fernández-Arroyo¹¹, Jorge Joven¹¹, Elsa Pérez¹², Margarita García¹, Neus Bosch¹, Eugeni López-Bonet¹², Samia Saidani¹, María Buxó¹¹, Javier A Menendez¹ and Begoña Martin-Castillo¹. ¹Catalan Institute of Oncology, Barcelona, Spain; ²Hospital Donostia, Donostia-San Sebastián, Spain; ³Hospital de Mataró, Barcelona, Spain; ⁴Vall d’Hebron Institute of Oncology (VHIO), Barcelona, Catalonia, Spain; ⁵Hospital Universitario de Canarias, La Laguna, Tenerife, Spain; ⁶Hospital Universitario de Salamanca, Salamanca, Spain; ⁷Sant Joan de Reus University Hospital, Reus, Spain; ⁸Hospital de Txagorritxu, Vitoria-Gasteiz, Araba, Spain; ⁹Hospital Universitario Central de Asturias, Oviedo, Spain; ¹⁰Girona Biomedical Research Institute, Girona, Spain; ¹¹Institut d’Investigació Sanitària Pere Virgili, Reus, Spain and ¹²Dr. Josep Trueta Hospital of Girona, Girona, Spain.

**Body: Background:** Epidemiological, pre-clinical, and window-of-opportunity trials have suggested that the anti-diabetic drug metformin (MET) may have beneficial effects on breast cancer (BC). The clinical relevance of combining MET with current standards of care for first-line treatment of BC is unknown. We investigated the safety and efficacy of adding MET to neoadjuvant chemotherapy plus trastuzumab (TTZ) in patients with HER2-positive early BC.

**Methods:** In a randomized, multicenter, open-label phase 2 study, women with operable, locally advanced, or inflammatory HER2+ BC were randomly assigned in a 1:1 ratio to receive daily MET (1,700 mg) for 24 weeks concurrently with 12 cycles of weekly paclitaxel plus TTZ followed by four cycles of fluorouracil, epirubicin, cyclophosphamide/3w plus TTZ (arm A) or equivalent sequential chemotherapy plus TTZ without MET (arm B), followed by surgery. Patients were stratified by age, extent of disease (cT2 cN0-1 vs ≥T3 or ≥N2), and hormone receptor (HR)-status. The intention-to-treat (ITT) safety population comprised all women who underwent randomization and received at least one treatment dose. Primary endpoint was the rate of pathological complete response (pCR) analyzed in the per-protocol (PP) efficacy population. pCR was defined as absence of invasive cancer in the breast and axillary nodes, irrespective of carcinoma in situ (ypT0/is ypN0). **Results:** From June 1, 2012 to March 17, 2016, 98 patients were assessed for eligibility at 10 centers in Spain. Of 84 (85.7%) patients who were randomized, 41 patients were allocated to arm A and 43 to arm B. The most common adverse effects (AEs) in the 79 ITT patients were fatigue, diarrhea, nausea, alopecia, sensory neuropathy, mucositis, neutropenia, and elevated AST/ALT. Most AEs were grades 1–2 (>90% in both arms). The most common AEs grade ≥3 were neutropenia (7/38 women in arm A and 5/41 women in arm B) and diarrhea (5 and 0, respectively). The number of serious AEs was 3 in arm A (none of them was deemed to be MET-related) and 2 in arm B. Patients were stratified by age, extent of disease (cT2 cN0-1 vs ≥T3 or ≥N2), and hormone receptor (HR)-status. The intention-to-treat (ITT) safety population comprised all women who underwent randomization and received at least one treatment dose. Primary endpoint was the rate of pathological complete response (pCR) analyzed in the per-protocol (PP) efficacy population. pCR was defined as absence of invasive cancer in the breast and axillary nodes, irrespective of carcinoma in situ (ypT0/is ypN0). **Results:** From June 1, 2012 to March 17, 2016, 98 patients were assessed for eligibility at 10 centers in Spain. Of 84 (85.7%) patients who were randomized, 41 patients were allocated to arm A and 43 to arm B. The most common adverse effects (AEs) in the 79 ITT patients were fatigue, diarrhea, nausea, alopecia, sensory neuropathy, mucositis, neutropenia, and elevated AST/ALT. Most AEs were grades 1–2 (>90% in both arms). The most common AEs grade ≥3 were neutropenia (7/38 women in arm A and 5/41 women in arm B) and diarrhea (5 and 0, respectively). The number of serious AEs was 3 in arm A (none of them was deemed to be MET-related) and 2 in arm B. At week 12, one (2.9%) patient in arm A and 6 (15%) in arm B exhibited asymptomatic decreases in the left ventricular ejection fraction (LVEF). At week 24, none (0%) of the patients in arm A and 3 (7.9%) patients in arm B presented decreases of LVEF below 50% and >10% from baseline. Only one patient (2.7%) in arm B experienced symptomatic heart failure. The rates of breast-conserving surgery were 79.3% and 58.6% in PP arms A and B, respectively. Also, 19/29 PP patients in arm A (65.5%, 95% CI 47–80) had a pCR versus 17/29 PP patients (58.6%, 95% CI 41–74) in arm B (OR 1.34 [95% CI 0.46–3.89], p=0.589). The combined rates of pCR and near-pCR (ypT1aN0) were 79.3% in arm A and 72.4% in arm B. Significantly fewer pCR were noted for tumors that were HR-positive regardless of which arm the patients were randomized. **Conclusion:** The higher pCR rate in HER2+ BC patients receiving MET-containing neoadjuvant therapy and TTZ did not reach statistical significance. Evaluation of long-term outcome data such as 5-year disease-free survival and correlative biological studies are needed to evaluate the clinico-molecular relevance of these findings.
A phase II study of copper-depletion using tetrathiomolybdate in patients with breast cancer at high risk for recurrence: Updated results


Background: Metals have emerged as a viable therapeutic target for a new generation of anti-cancer and anti-metastatic agents. Copper, an essential trace element, serves as an important catalytic cofactor in several biological functions and has emerged as an essential factor in carcinogenesis. Among other elements, bone marrow derived VEGFR2+ endothelial progenitor cells (EPCs) and copper-dependent lysyl oxidase (LOX) are key elements in tumor progression. We hypothesized tetrathiomolybdate (TM)-associated copper depletion (CD) inhibits tumor metastases by reducing the number of EPCs and other copper dependent processes in the pre-metastatic niche. These results are an update of our previously reported study (Chan N, Willis A, Kornhauser N et al. Influencing the Tumor Microenvironment: Phase 2 Study of Copper Depletion with Tetrathiomolybdate in High Risk Breast Cancer and Preclinical Models of Lung Metastases. Clin Cancer Res. October 21, 2016) with longer follow-up.

Methods: A single arm phase II study of breast cancer (BC) patients (pts) at high risk for recurrence, defined as node+ triple negative (TNBC), stage 3 and 4 with no evidence of disease (NED) were enrolled on a trial of CD with TM. TM was given to maintain ceruloplasmin (Cp) levels between 8-16 mg/dl for two years with an extension phase or until relapse. The primary endpoint was a change in EPCs measured by flow cytometry before and during treatment. Secondary endpoints included tolerability, safety, PFS and LOXL-2 levels.

Results: Seventy-five pts received 2778 cycles of TM on the primary and extension study. The primary study treatment duration was 24 cycles (each cycle is 28 days) plus an extension phase. The median age is 51 years (range 29-66). Forty-five pts have stage 2/3 BC and 30 with stage 4 NED. Forty-eight percent of pts are TNBC and 40% of pts are stage 4 NED. Median Cp levels were monitored with each cycle. A decrease from 28 to 16 (p<0.0001) was seen after one cycle. Interestingly, TNBC pts seemed to have a greater decrease from 23.5 to 13 after one cycle. TM was well tolerated with grade 3/4 toxicities including: reversible neutropenia (2.3%), febrile neutropenia (0.04%), fatigue (0.2%). Five-year analysis showed a decrease in EPC's (p=0.004) and LOXL-2 (p<0.001). At a median follow-up of 7.1 years, the EFS for 75 pts is 71.4%. The EFS for 36 pts with TNBC is 71.7%. EFS for stage 2/3 TNBC is 83% and for stage IV TNBC is 59.3%.

Conclusions: TM is safe, well tolerated and appears to affect multiple components of the tumor microenvironment that have been identified in pre-clinical models as important for progression. Ongoing studies in banked specimens are underway to further delineate its effect on copper dependent processes necessary for metastases. Randomized trials are warranted, especially in patients who are at high risk for relapse such as those with TNBC.
Title: Precision therapeutic combinations are synergistic against triple negative breast cancer using compensatory pathways

Jeffrey Solzak¹, Brad Hancock¹, Robin Paul¹ and Milan Radovich¹. ¹Indiana University School of Medicine, Indianapolis, IN.

Body: Introduction: Triple negative breast cancer (TNBC) accounts for 15% of all breast cancer cases in the United States, and despite its lower incidence, contributes to a disproportionately higher rate of morbidity and mortality compared to other breast cancer subtypes. In an effort to treat TNBC, a cancer that has no targeted therapies, many have chosen to experiment with combinations of drugs that are chosen for their genomic expression or “hard targets”. It has been previously noted however, that single agent therapeutics can change the genomic landscape of both mice and human cells and may be responsible for resistance. Here we show that targeting a compensatory pathway after treatment with a single therapy, results in synergistic combinations and can outperform the choice of hard target therapies.

Methods: Eight TNBC cell lines were chosen based on their abundance of clinically actionable targets. The primary hard target combinations were chosen using data from TCGA and a board consisting of oncologists and researchers at Indiana University School of Medicine. Compensatory therapies were found using RNA sequencing data from untreated versus single therapeutic treated TNBC cell lines. The merged transcript RPKMs were transformed and analyzed for differential expression. Statistically significant genes were imported into Ingenuity Pathway Analysis (IPA) to identify either therapeutics or genomic targets using the Causal network analysis and Upstream regulator functions. All drug combinations were tested in their respective cell lines and cell viability was assessed via Celltiter-Fluor. Synergy of the combinations was calculated using the Chou-Talalay method.

Results: Using genomically chosen hard targets for drug combinations, all eight cell lines displayed additive or antagonistic results except low nanomolar doses for the MDA-MB-231 cell line. Dosing of MDA-MB-231s with Debrafinib and Pazopanib however, turned highly antagonistic as dosing increased. Using next-generation RNA sequencing data of TNBCs, IPA analysis identified several compensatory targets for each cell line when treated with one of the primary genomically driven drug at its IC50. Using dose escalation of the new drugs with a single hard target drug, we found that each compensatory combination displayed a striking increase in synergy across all TNBC cell lines treated when compared to their original hard target combination.

Conclusion: RNA sequencing of TNBC cells lines treated with single therapies chosen by actionable genomic landscape has revealed compensatory pathways, indicating further druggable targets. These compensatory pathways have been observed to more efficacious in treating TNBC cell lines. Using therapeutics that are either FDA approved or in clinical trials we have found that each compensatory combination shows a higher level of synergy across all cell lines. These data show that choosing a secondary therapy based on compensatory pathways may outperform hard target combinations in the clinic.
Title: Elacestrant, a novel oral selective estrogen receptor degrader (SERD), decreases tumoral \(^{18}\text{F}\)-FES uptake in a phase 1 study of ER+, HER2-, advanced breast cancer patients

Elisabeth GE de Vries, Clasina M Venema, Andor WJM Glaudemans, Agnes Jager, C Willemien Menke-van der Houven van Oordt, Patrick Neven, Hai Jiang, Dannie Wang, Alison O'Neill, Abhay Patki and Philippe Aftimos. 1University Medical Center Groningen, Groningen, Netherlands; 2Erasmus MC Cancer Institute, Rotterdam, Netherlands; 3VUMC Medical Center & Cancer Center Amsterdam, Amsterdam, Netherlands; 4UZ KU-Leuven, Belgium; 5Radius Health, Inc., Waltham, MA and 6Institut Jules Bordet – Universite Libre de Bruxelles, Brussels, Belgium.

Body: Background: The estrogen receptor (ER) expressed in approximately 70% of breast cancers, can be mapped using \(^{18}\text{F}\)-\(\alpha\)-\[^{18}\text{F}\]Fluoro-17\(\beta\)-estradiol (FES)-PET, a non-invasive molecular whole body imaging tool capable of assessing ER target engagement by endocrine therapy. Elacestrant (RAD1901) is a novel, non-steroidal oral SERD that has demonstrated single-agent activity in heavily pre-treated patients with ER+ advanced breast cancer (Bardia et al., J Clin Oncol 35, 2017: suppl; abstr 1014).

The primary objective of this study was the visualization and quantification of residual ER-binding with FES-PET after treatment with elacestrant.

Methods: In the RAD1901-106 phase 1 study (NCT02650817), patients with advanced ER+/HER2 - breast cancer were treated with elacestrant at two dose levels (200 mg or 400 mg qd orally) for the first 14 days. Tumoral FES uptake was measured at baseline and day 14. After FES-PET scan at day 14, all patients received elacestrant 400 mg qd until disease progression or discontinuation due to another cause. Key inclusion criteria included postmenopausal women with advanced ER+/HER2- breast cancer, who have progressed after \(\geq\) 6 months of at least 1 line of endocrine treatment in the metastatic setting, with measurable disease according to RECIST criteria v1.1 or clinically evaluable disease. The PET scan was performed 60 min after intravenous injection of 200 MBq FES. Maximum standardized uptake values (SUVmax) were calculated and corrected for background activity. FES positive lesions had a SUVmax >1.5. FES response was defined as a \(\geq\) 75% median decrease of SUVmax between the two scans. ESR1 mutation status was determined from circulating tumor DNA collected at multiple time points.

Results: Twenty-four patients were screened and 16 patients were enrolled (8 at each dose level). Median lines of prior therapy were 3, with 6 (38%) patients having previously received fulvestrant and 8 (50%) patients harboring at least 1 ESR1 mutation at baseline. Elacestrant demonstrated a median of 88% (range 59-97.1%) reduction in tumorFES uptake following 14 days of treatment. Eleven out of 16 patients had >75% reduction in FES uptake at day 14. At the data cutoff date of 2 May 2017, median treatment duration was 8.1 weeks and 8 (50%) patients remained on study. Elacestrant was generally well-tolerated, with the most common treatment-related adverse events (CTCAE v4.03) being low grade nausea (gr1 = 50%; gr2 =19%; gr3/4 = 0%), dyspepsia (gr1 = 25%; gr2 = 19%; gr3/4 = 0%) and dysphagia (gr1 = 19%; gr2 =12%; gr3/4 = 0%). Updated analyzes with mature data on correlation of FES uptake reduction and tumor response, treatment duration, safety and ESR1 mutation will be presented.

Conclusions: Elacestrant significantly reduced FES uptake at both 200 mg and 400 mg doses after 14 days of treatment in heavily pre-treated patients with advanced ER+/HER2- breast cancer, including those harboring ESR1 mutations.
Title: ICEC0942, a new oral selective inhibitor of the cell cycle and transcriptional regulator CDK7 for the treatment of estrogen receptor positive and negative breast cancer


Body: CDK7 is remarkable as a key regulator of both cell cycle progression and gene expression. CDK7 promotes cell cycle progression by phosphorylating cell cycle CDKs in the T-loop, thus stimulating their activities. Additionally, phosphorylation of RNA polymerase II (PolII) by CDK7 is required for transcription initiation. Deregulation of cell cycle and transcription processes is common to most cancer types, so CDK7 inhibitors offer considerable promise as cancer therapeutics.

We previously reported the identification of the first selective CDK7 inhibitor, BS-181, and demonstrated its ability to inhibit breast cancer cell growth in vitro and in vivo (Ali et al 2009 Cancer Res). Screening of more than one thousand analogues has allowed development of a clinical candidate CDK7 inhibitor, named ICEC0942. ICEC0942 selectively inhibits CDK7 with an IC$_{50}$ of 40nM. In vitro analyses reveal that ICEC0942 inhibits hormone receptor positive and triple-negative breast cancer cell lines, with GI$_{50}$ values ranging between 0.2-0.3 µM. Growth inhibition is accompanied by inhibition of CDK7 targets, including CDK1, CDK2 and PolII phosphorylation. In xenograft studies using several cancer cell lines, the drug shows substantial anti-tumor effects, with a notable lack of toxicity at efficacious doses. In the combination setting with tamoxifen, ICEC0942 completely blocks growth of ER-positive tumor xenografts, indicative of potential for co-treatment with hormonal agents.

Extensive ADMET and PK/PD studies confirm the suitability of ICEC0942 as a cancer drug and have shown that ICEC0942 is orally bioavailable. Moreover, xenograft tumor studies have allowed definition of surrogate biomarkers of tumor response. Taken together, our findings confirm CDK7 as an important drug target for ER-positive and -negative breast cancer and identify ICEC0942 as a prototype drug with utility as a single agent or in the combination setting. Our findings also point to the potential value of CDK7 inhibition by ICEC0942 in other cancer types that have characteristics of transcription factor addiction and/or cell cycle deregulation.

Development of ICEC0942 was made possible through funding by EPSRC, Cancer Research UK and Cancer Research Technologies.
Title: A randomized phase II trial evaluating the endocrine activity and efficacy of neoadjuvant degarelix versus triptorelin in premenopausal patients receiving letrozole for primary endocrine responsive breast cancer (TREND; IBCSG 41-13)

Marco Colleoni1, Kathryn Gray1, Elisabetta Munzone1, Silvia Dellapasqua1, Claudio Zamagni1, Lorenzo Gianni1, Harriet Johansson1, Giuseppe Viale1, Roswitha Kammler1, Rudolf Maibach1, Manuela Rabaglio-Poretti1, Angelo Di Leo1, Alan S Coates1, Richard D Gelber1, Meredith M Regan1 and Aron Goldhirsch1. 1International Breast Cancer Study Group.

Body: Background: Neoadjuvant endocrine therapy (NET) with gonadotropin-releasing hormone (GnRH) agonist and aromatase inhibitors is effective in selected premenopausal patients (pts). Degarelix, an antagonist of GnRH, has immediate onset of action through binding to GnRH receptors in the pituitary gland and thereby suppressing the production of LH and FSH. Its suppressing activity in premenopausal women might be faster and free of estrodial breakthrough on continued treatment compared with a GnRH angonist, and thereby provide significant clinical value for pts who are candidates for short-term NET.

Methods: Eligible pts were premenopausal women with cT2-4b, any nodal stage, ER and PgR >50%, HER2-negative (by IHC and/or ISH) breast cancer who were not candidates for breast conserving surgery. Premenopausal status was determined locally with estradiol (E2) levels >54 pg/mL (or >198 pmol/L), measured within 14 days prior to randomization. Pts were randomized 1:1 to Triptorelin (T) 3.75 mg i.m. on day 1 of every cycle or Degarelix (D) 240 mg s.c. given as two injections of 120 mg on day 1 of cycle 1, then 80 mg s.c. on day 1 of cycles 2-6 with letrozole (L) 2.5 mg/day for 6 cycles. Each cycle was 28 days. Definitive surgery was performed within 2-3 weeks after the last administration of T or D. Serum was collected prior to the first injection (baseline), 24 and 72 hours, 7 and 14 days, then prior to injection on day 1 of cycles 2-6. The primary endpoint was time to optimal ovarian function suppression (OFS) calculated as time from the first injection of D or T to the first assessment of centrally assessed 17-β-estradiol (E2) level in the range of optimal OFS (≤2.72 pg/mL or ≤10 pmol/L) during the 6 cycles of NET. The trial had 90% power to detect a difference using a logrank test, 2-sided α=0.05. Secondary endpoints included tolerability, Ki67 changes, PEPI score, best overall response. NCT02005887

Results: TREND completed accrual of 51 pts in January 2017. A preliminary analysis based on the first 45 pts is reported here. 89% of patients were ≥40 yrs, 76% had T1-2 and 22% T3 tumors, and 51% were node-positive. Dominant histology type was ductal (93%). The table summarizes centrally-assessed E2 according to treatment at baseline and for the first 5 assessment time points indicating immediate suppression for the D+L arm. E2 levels on day 1 of cycles 2-6 were all below the limit of quantification (0.625 pg/mL) for the D+L arm. For the T+L arm continued OFS was not maintained in 4 pts.

<table>
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<tr>
<th></th>
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<th>Cycle 2</th>
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<tbody>
<tr>
<td>Day:</td>
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<td>1</td>
<td>3</td>
</tr>
<tr>
<td>No. Pts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D+L</td>
<td>22</td>
<td>22</td>
<td>21</td>
</tr>
<tr>
<td>T+L</td>
<td>23</td>
<td>23</td>
<td>21</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>D+L</td>
<td>96.2 (64.2,206.8)</td>
<td>10.1 (4.0,21.8)</td>
<td>0.6 (0.6,1.0)</td>
</tr>
<tr>
<td>T+L</td>
<td>85.1 (49.7,118.0)</td>
<td>37.4 (17.9,59.2)</td>
<td>12.8 (7.7,23.8)</td>
</tr>
</tbody>
</table>

Conclusion: Evidence from this first analysis demonstrates rapid and maintained OFS with the combination of D+L as a NET in premenopausal breast cancer patients. The final analysis of the total population, including secondary endpoints, will be presented at the symposium.
2017 San Antonio Breast Cancer Symposium

Publication Number: P1-10-07

Title: Efficacy and safety results from a randomized, phase II study of CC-486 in combination with fulvestrant in postmenopausal women with estrogen receptor–positive (ER+), human epidermal growth factor receptor 2–negative (HER2−) metastatic breast cancer (MBC)

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Body: Background: Most patients diagnosed with breast cancer have ER+ tumors. Treatment of ER+ MBC typically involves endocrine therapy, including aromatase inhibitors and selective ER modulators such as tamoxifen; however, many patients develop resistance. Fulvestrant, an ER antagonist, is a commonly prescribed second- or third-line therapy for postmenopausal patients who have progressed on endocrine therapy; although, most patients will eventually develop resistance to this drug as well. It was hypothesized that CC-486, an oral formulation of azacitidine, may resensitize patients to endocrine therapy and possibly delay resistance to fulvestrant through the epigenetic regulation of certain genes.

Methods: 97 postmenopausal female patients aged ≥ 18 years with ER+, HER2− MBC refractory to an aromatase inhibitor were randomized 1:1 to receive CC-486 300 mg on days 1 through 21 and fulvestrant 500 mg on days 1 and 15 of cycle 1 and day 1 of subsequent 28-day cycles or the same fulvestrant regimen alone. The primary endpoint was progression-free survival (PFS) based on investigator’s assessment using RECIST version 1.1 and summarized by the Kaplan-Meier method. A Cox proportional hazards model was used to estimate the hazard ratio (HR; including a 2-sided 95% CI), and a log-rank test was used to calculate P values for comparisons between treatment arms. Key secondary endpoints included objective response rate (ORR), overall survival (OS), and safety.

Results: 48 patients were included in the CC-486 + fulvestrant arm and 49 in the fulvestrant-alone arm. Median age was 63 years. Baseline characteristics were generally balanced between treatment groups, with some exceptions. The CC-486 + fulvestrant treatment cohort had fewer patients aged ≥ 65 years (40% vs 49%), with an ECOG PS of 1 (25% vs 57%), or with liver metastases (29% vs 43%) than did the fulvestrant-alone cohort. At the time of this analysis, 36 patients (75%) in the CC-486 + fulvestrant arm and 40 patients (82%) in the fulvestrant-alone arm had discontinued treatment, mostly due to progressive disease (81% and 90%, respectively). Median PFS was 5.5 months in both treatment groups (HR 0.87; 95% CI, 0.54 - 1.42; P = 0.599). ORR was 8.3% vs 2.0% in patients receiving CC-486 + fulvestrant vs fulvestrant alone, respectively. Median OS has not been reached. In patients who received CC-486 + fulvestrant, the most common any-grade nonhematologic treatment-emergent adverse events (TEAEs) were nausea (78%), vomiting (78%), diarrhea (44%), and constipation (41%), and the most frequent any-grade hematologic TEAE was neutropenia (26%). Of patients who discontinued due to AEs, most patients receiving CC-486 + fulvestrant treatment discontinued due to gastrointestinal (GI) TEAEs.

Conclusion: The addition of CC-486 to fulvestrant did not improve PFS in patients with ER+, HER2− MBC compared with fulvestrant alone, and GI TEAEs were reported in a majority of patients. These results do not support further evaluation of this combination in this setting.
Title: Development of a first-in-class oral selective ERα covalent antagonist (SERCA) for the treatment of ERα<sup>WT</sup> and ERα<sup>MUT</sup> breast cancer

Manav Korpal<sup>1</sup>, Xiaoling Puyang<sup>1</sup>, Craig Furman<sup>1</sup>, Guo Zhu Zheng<sup>1</sup>, Deepi Banka<sup>1</sup>, Jeremy Wu<sup>1</sup>, Zhaojie Zhang<sup>1</sup>, Michael Thomas<sup>1</sup>, Crystal Mackenzie<sup>1</sup>, Huilan Yao<sup>1</sup>, Victoria Rinkunas<sup>1</sup>, Pavan Kumar<sup>1</sup>, Benjamin Caleb<sup>1</sup>, Craig Karr<sup>1</sup>, Vanitha Subramanian<sup>1</sup>, Sean Irwin<sup>1</sup>, Nicholas Larsen<sup>1</sup>, Frederic Vaillancourt<sup>1</sup>, Tuong-Vi Nguyen<sup>1</sup>, Allison Davis<sup>1</sup>, Betty Chan<sup>1</sup>, Ming Hong Hao<sup>1</sup>, Morgan O'Shea<sup>1</sup>, Sudeep Prajapati<sup>1</sup>, Sergei Agoulnik<sup>2</sup>, Galina Kuznetsov<sup>2</sup>, Namita Kumar<sup>2</sup>, Yanke Yu<sup>2</sup>, George Lai<sup>2</sup>, Andrew Hart<sup>2</sup>, Sean Eckley<sup>2</sup>, Peter Fekkes<sup>1</sup>, Todd Bowser<sup>1</sup>, Julie J Joshi<sup>1</sup>, Anand Selvaraj<sup>1</sup>, Suzanne Wardell<sup>2</sup>, John Norris<sup>3</sup>, Sherri Smith<sup>1</sup>, Dominic Reynolds<sup>1</sup>, Lorna Mitchell<sup>1</sup>, John Wang<sup>1</sup>, Lihua Yu<sup>1</sup>, Amy Kim<sup>1</sup>, Nathalie Rioux<sup>1</sup>, Tarek Sahmoud<sup>1</sup>, Markus Warmuth<sup>1</sup>, Peter G Smith<sup>1</sup> and Ping Zhu<sup>1</sup>. <sup>1</sup>H3 Biomedicine, Inc., 300 Technology Square, Cambridge, MA; <sup>2</sup>Eisai Inc., 4 Corporate Drive, Andover, MA and <sup>3</sup>Duke University, Research Drive, LSRC Bldg, C251, Durham, NC.

Body: Mutations in estrogen receptor alpha (ERα) are detected in up to 30% of breast cancer patients who have relapsed during endocrine therapy. ERα mutations functionally confer resistance to existing classes of endocrine therapies, likely through gaining constitutive activity. The fact that current ER-directed therapies are only partially effective in the ERα mutant setting, and that a significant proportion of resistant breast cancer metastases continue to remain dependent on ERα signaling for growth/survival, highlights the critical need to develop the next generation of ERα antagonists that can overcome aberrant ERα activity. Using structure-based drug design approaches we have identified a novel class of ERα antagonist referred to as Selective ERα Covalent Antagonist (SERCA) that inactivate both wild-type and mutant ERα by targeting a unique cysteine residue that is not conserved among other steroid hormone receptors. Biophysical, biochemical and cellular analyses confirm the covalent mechanism of action, specific binding to ER and selective inhibition of ERα-dependent transcription of SERCAs. H3B-6545 is a highly selective SERCA that potently antagonizes wild-type and mutant ERα in biochemical and cell based assays demonstrating increased potency over standard of care and other experimental agents. In vivo, H3B-6545 shows superior efficacy to fulvestrant in the MCF-7 xenograft model with once daily oral dosing, achieving maximal antitumor activity at doses >10x below the maximum tolerated dose in mice. In addition, H3B-6545 shows superior antitumor activity to both tamoxifen and fulvestrant in patient derived xenograft models of breast cancer carrying estrogen receptor mutations. In summary, H3B-6545 is a first-in-class, orally available and selective ER covalent antagonist with a compelling pre-clinical profile that is being developed for the treatment of ERα positive breast cancer.
Title: EPHA2-targeting enhances eicosapentaenoic acid cytotoxicity against triple-negative inflammatory breast cancer via ABCA1 inhibition–mediated membrane rigidity


Background: Effective treatment options for triple-negative inflammatory breast cancer (TN-IBC), the most aggressive form of breast cancer, are currently lacking. We previously reported that mediators of inflammation promote the growth of TN-IBC xenografts. Eicosapentaenoic acid (EPA), an omega-3 fatty acid (fish oil) with anti-inflammatory properties, is an emerging FDA-approved therapeutic with a favorable toxicology profile. Here we aimed to develop a novel approach to enhance EPA efficacy against TN-IBC by identifying a kinase inhibitor that synergizes with EPA's antitumor activity.

Methods and Results: Using a high-throughput siRNA screen in the TN-IBC cell line SUM149PT, we identified inhibition of ephrin type-A receptor 2 (EPHA2), an oncogenic receptor tyrosine kinase, as a target that sensitizes TN-IBC cells to EPA therapy. To determine the clinical relevance of EPHA2, we investigated a meta-analysis of breast cancer mRNA expression data sets and found that high EPHA2 tumor expression, compared with low expressing, correlated significantly with poor overall survival in TN-IBC patients (P = 0.01), while not with other subtypes. Similar findings were observed in vitro, were EPHA2 protein and mRNA overexpression occurred predominantly in the TN subtypes among 49 and 51 breast cancer cell lines (63% and 47%, respectively), highlighting EPHA2 translational potential. Functional expression studies using proliferation and apoptosis assays in vitro, and xenografts in vivo, were performed in two EPHA2-expressing TN-IBC cell lines, SUM149PT and BCX010, to validate EPHA2 as a synergistic combinational target with EPA. EPHA2 gene silencing in combination with EPA significantly reduced cell growth, and enhanced apoptosis, compared with untreated and monotherapy in vitro (P < 0.05), and in vivo (P < 0.001). To translate our findings to the clinic, we validated dasatinib, an FDA-approved small molecule inhibitor of EPHA2, in combination to EPA to significantly enhance apoptosis of TN-IBC cells in vitro (P < 0.05) and in vivo (P < 0.05), compared with untreated and monotherapies. Using membrane fluidity assessment and cholesterol quantification we determined that apoptosis induction after combination therapy was due to increased membrane rigidity and cholesterol concentrations in the plasma membrane of TN-IBC cells (P < 0.05, compared with monotherapies). Finally, we discovered by western blot and gain/loss-of-expression studies that combination therapy inhibited the cholesterol efflux protein ATP-binding cassette sub-family A member 1 (ABCA1), which plays a significant role mediating increased cellular cholesterol (P < 0.05), cell membrane rigidity (P < 0.05), and induction of apoptosis (P < 0.05) in TN-IBC after EPA and EPHA2-targeting combination therapy.

Conclusions: This is the first study demonstrating that EPA can enhance conventional targeted therapy against breast cancer. Our study provides molecular and preclinical evidence to support the development of an EPA/EPHA2-inhibition–based phase I clinical trial for patients with EPHA2-positive TN-IBC; our study further suggests the use of EPHA2 and ABCA1 protein expression as biomarkers for patient selection and therapeutic response.
Title: Tumor infiltrating lymphocytes (TILs) among high risk for recurrence breast cancer patients treated with tetrathimolybdate (TM)

Marissa D Rybstein¹, Eleni Nackos¹, Naomi Kornhauser¹, Tessa Cigler¹, Eleni Andreopoulou¹, Anne Moore¹, Marta Cobham¹, Veronica Fitzpatrick¹, Sandra Demaria¹ and Linda T Vahdat¹. ¹New York Presbybyterian - Weill Cornell Medical Center, New York, NY.

Body: Background: Tumor infiltrating lymphocytes (TILs) evaluated in the primary tumor biopsy or surgical resection have been well established as having prognostic significance in patients with triple negative breast cancer (TNBC) and HER2+ breast cancer treated with adjuvant chemotherapy (Savas et al, Nat Rev Clin Oncol 2016). In TNBC, stromal TILs behave as a continuous variable with every 10% increase in TIL resulting in a decrease in risk of recurrence and death. The definition of lymphocyte-predominant breast cancer (LPBC) has been used for tumors that contain 50%–60% TILs and usually have a particularly good outcome (Salgado et al, Ann Oncol 2015). Our group recently demonstrated in a phase II single arm study that tetrathimolybdate (TM), a copper-depleting agent, resulted in improved event free survival (EFS) for TNBC patients compared to historical controls. The 2-year event-free survival (EFS) for stage 2-3 and stage 4 NED was 91% and 67%, respectively. In this analysis, our goal was to explore whether the encouraging results we observed were influenced by enrolling TNBC patients with better prognostic factors at initial diagnosis, namely higher stromal TIL score, in our copper depletion trial.

Methods: Archived primary breast tissue was available from 67 of the 75 patients enrolled in the phase II TM trial. The phase II study included patients with stage II TNBC or stage III or IV NED breast cancer patients, who were treated with TM for 2 years or until relapse. Here we focused on the 30 patients with TNBC. The demographic data for the patients is included in the following table.

Patient Demographics

<table>
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<tr>
<th>Age at diagnosis</th>
<th>Stage at study entry</th>
<th>Prior Adjuvant or Neoadjuvant therapy</th>
<th>Number of prior chemotherapy regimens in metastatic setting</th>
<th>%Tumor Infiltrating Lymphocytes</th>
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</table>

The number of TILs in each sample was calculated by an experienced pathologist using published criteria (Salgado et al, Ann Oncol 2015). We used TILs >50% to define LPBC.

Results: Overall, we found that only 3/30 (10%) of TNBC patients had TILs >50%. In addition, 14/30 (46.7%) of TNBC patients had tumors with <10% TILs. The 2-year EFS for the patients with TILs >10% v. <10% was 76.9% v. 69.8%, respectively. (P=0.65)

Conclusions: Only 10% of TNBC patients enrolled in the study had LPBC at diagnosis thus indicating that this cohort was not enriched for patients with immunogenic tumors. When stratified by TILs >10% or <10%, there was no statistically significant difference in EFS. Although the analysis is limited due to the small sample size, it does suggest that the amount of TILs present at initial diagnosis did not influence the overall outcome for patients treated with TM.
Title: Multivalent exposure of trastuzumab on iron oxide nanoparticles enhances antitumor activity and weakens drug resistance in HER2+ breast cancer cells

Marta Truffi¹, Matteo Monieri¹, Luca Sorrentino¹, Serena Mazzucchelli¹, Miriam Colombo², Laura Pandolfi², Davide Prosperi² and Fabio Corsi¹,³. ¹University of Milan, “L. Sacco”, Milan, Italy; ²University of Milan-Bicocca, Milan, Italy and ³ICS Maugeri S.p.A., Pavia, Italy.

Body: **Background:** The identification of new strategies aimed to optimize the treatment of breast cancer and its metastases represents a great technical and medical challenge. Target-specific therapies, such as Trastuzumab (TZ), have revolutionized the clinical scenario in certain subsets of cancer. However, the huge variability in response to therapy and the frequent onset of drug resistance in patients still hamper the therapeutic success. Antibody-conjugated nanoparticles may combine specific recognition of tumor cells with the capability to act as innovative reservoir of active drugs. Here, multivalent TZ-conjugated colloidal nanoparticles were developed as target-specific and biologically active nanosystem to enhance the therapeutic potential toward HER2+ breast cancer.

**Methods:** Iron oxide nanoparticles conjugated with multiple half chains of TZ have been developed and tested in different HER2+ breast cancer cell lines, in comparison to free TZ or untargeted nanoparticles. Active targeting and specificity toward HER2 receptor was assessed by flow cytometry and confocal microscopy. Cellular uptake of nanoparticles and HER2 endocytosis were followed by electron or confocal microscopy. Direct anticancer efficacy was assessed by incubation of free or nanoformulated TZ on sensitive breast cancer cells, and analysis of cell viability, cell cycle, and expression of p27kip1. Finally, nanoparticles were tested on TZ-resistant breast cancer cell lines for capability of re-sensitization.

**Results:** TZ-conjugated nanoparticles showed specific targeting of HER2, with induction of site-specific phosphorylation in the catalytic domain of the receptor and cellular uptake by endocytosis. Treatment with TZ-conjugated nanoparticles dramatically decreased cancer cell viability, by significantly improving the antitumor activity of TZ. This effect was independent from the ADCC mechanism, and associated with marked induction of p27kip1 expression and cell cycle arrest in G1 phase in TZ-sensitive SKBR-3 cells. TZ-conjugated nanoparticles also affected viability of breast cancer cells insensitive to TZ, further confirming enhanced potential of the nanoformulation and suggesting interference with some mechanisms of resistance.

**Conclusions:** Our results provide evidence that multivalent exposure of TZ half chain on iron oxide nanoparticles affords enhanced antitumor potential and target-specific activity in HER2+ breast cancer cells. Powerful inhibition of HER2 signaling by TZ-conjugated nanoparticles could favor responsiveness of drug resistant cells, thus suggesting novel therapeutic strategies to overcome resistance.
Differential benefit of intra-operative administration of ketorolac on breast cancer disease recurrence according to baseline body mass index

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Body: Background:
According to the last estimates, 63% of the women in the US are either overweight or obese. Increased body mass index (BMI) has been recognized as a risk factor for developing breast cancer (BC) and associated with adverse survival. So far, few treatment modalities focusing on the biological features of BC patients with increased adiposity have been evaluated. In cancer patients, elevated leptin levels have been found, among others, to stimulate the Rho GTPase pathway, involved in cell adhesion and motility. Here, using a unique retrospective institutional cohort including a total of 847 BC patients, we aimed at assessing whether the intra-operative administration of ketorolac, a Non-Steroidal Anti-Inflammatory Drug (NSAID), with a recently documented Rho GTPase inhibitory activity, would be associated with an improvement in distant disease recurrence according to BMI. We further assessed in an independent series of 1,009 patients, the effect of intra-operative diclofenac, another NSAID without Rho GTPase inhibitory activity.

Patients and methods:
In the institutional retrospective and consecutive 'ketorolac' series of patients with primary BC surgery, 538 were treated with and 309 without a single-dose (typically 20 mg in patients under 60 kg and 30 mg in patients ≥60 kg) of intra-operative ketorolac. In the 'diclofenac' series, 789 patients were treated with intra-operative diclofenac (75 mg) and 220 without. Competing risk analysis of distant recurrences was done by crude cumulative incidence curves to be interpreted as the cumulative probability of distant metastases as first event and consistently by Fine & Gray semi-parametric models on sub-distribution hazards. Subgroup analyses were conducted according to the Dixon&Simon approach. These analyses were adjusted for standard clinico-pathological variables: NSAID use, tumor size, age, nodal status, grade, ER status, and BMI. The median follow-up time of the ketorolac and diclofenac cohort was 5.7 and 8.0 years, respectively.

Results:
In both cohorts, the administration of the NSAID was associated with younger age at diagnosis, consistent with previous reports. The administration of ketorolac was associated with decreased incidence of distant recurrences both in the unadjusted and adjusted analysis (HRadj= 0.55, 95%CI: 0.35-0.86, p=0.01). Subgroup analyses in patients with BMI<25 and ≥25 (overweight and obese) revealed that the effect was limited to the high BMI group of patients both in the unadjusted and adjusted analysis (HRadj= 0.58, 95%CI: 0.33-0.98). The administration of diclofenac was not associated with decreased incidence of distant recurrences, nor in the global population, neither in the BMI subgroups.

Conclusion:
The fact that only ketorolac but not diclofenac was associated with a significant reduction of distant recurrences in BC patients with elevated BMI could be explained by the specific Rho GTPase inhibitory effect of the R-enantiomer of ketorolac, absent in diclofenac. While this study is limited by its retrospective nature, it suggests a potentially important repositioning of ketorolac in the intra-operative treatment of BC patients with elevated BMI. A prospective study is on its way.
Title: Olaparib nanoformulation in H-ferritin as a promising option for both BRCA-mutated and sporadic triple negative breast cancer: An in vitro study

Serena Mazzucchelli¹, Marta Truffi¹, Luca Sorrentino¹, Michela Bellini², Maria A Rizzuto², Roberta Ottria¹, Pierangela Ciuffreda¹, Davide Prosperi³ and Fabio Corsi¹,³. ¹University of Milan, Milan, Italy; ²University of Milan-Bicocca, Milan, Italy and ³Istituti Clinici Scientifici ICS Maugeri Pavia Spa SB, Pavia, Italy.

Body: Background: PolyADP-ribose polymerase (PARP) inhibitors are a novel promising strategy toward triple-negative breast cancer (TNBC), which often shows genomic instability or BRCA mutations. However, clinical results are controversial, and no benefits were demonstrated in case of wild type BRCA, possibly due to poor bioavailability, and inadequate nuclear delivery. Nanotechnology could overcome these major limitations. The aim of this study was to assess the anticancer efficacy of H-Ferritin nanoformulated Olaparib (HOla) vs. free Olaparib (Ola) on BRCA-mutated and non mutated TNBC cells.

Methods: BRCA-mutated HCC1937 cells and BRCA-wild type MDA MB-231 and MDA MB-468 cells were treated with HOla or free Ola in vitro. Active targeting and binding capability of HOla toward transferrin receptor 1 (TfR-1), over-expressed on TNBC cells, was assessed by flow cytometry. Internalization and intracellular localization of Ola and HOla was assessed by confocal microscopy. Anticancer efficacy was assessed by administration of increasing doses of HOla or Ola, comparing cell viability, cell cycle, cell death, PARP-1 cleavage and DNA damage. Finally, anti-PARP efficacy and proportion of drug in the nuclear compartment were compared between treatments.

Results: All TNBC cell lines over-expressed TIR-1 and were successfully recognized by HOla. Confocal microscopy showed a fast internalization of nanoparticles into cells, with intracellular persistence up to 48h. A marked increase in nuclear concentration of drug was observed with HOla compared to Ola, due to a strongly improved nuclear delivery by H-Ferritin mediated by a self-triggered mechanism. No significant antiproliferative effect was demonstrated with Ola at 10 nM, 50 nM or 100 nM. Conversely, HOla at 50 nM and 100 nM showed a 1000-fold higher anticancer activity in all TNBC cell lines. A possible contribution in cytotoxicity by H-Ferritin nanovector itself was excluded treating cells with void nanoparticles. Proportions of cell cycle arrest in G2/M, cell death, cleaved PARP-1 and DNA damage in terms of phosphorylated histone H2A.X were higher in HOla treated samples than in ones treated with free Ola.

Conclusions: Our findings suggest that nanoformulation of Ola strongly enhances cytotoxic efficacy of PARP inhibition as a stand-alone therapy, on both BRCA-mutated and wild type TNBCs allowing a targeted delivery into TNBC cells and a prompt homing into the nuclear compartment.
Title: Abemaciclib tablet formulation is bioequivalent to capsules

P Kellie Turner1, Jill C Chappell1, Aktham Aburub1, Wee Teck Ng1, Wei Zhang1, Jane Royalty2 and Palaniappan Kulanthaivel1. 1Eli Lilly and Company, Indianapolis, IN and 2Covance Early Clinical Development, Madison, WI.

Body: Introduction: Abemaciclib is being developed for the treatment of cancer, including advanced and metastatic breast cancer. Tablet formulations, which are smaller than the capsules used in the pivotal MONARCH 1, 2 and 3 breast cancer studies, have recently been developed. This study evaluated the bioequivalence of abemaciclib capsule and tablet formulations following single 150-mg doses, and the effect of food on the pharmacokinetics (PK) of abemaciclib following dosing with a 150-mg tablet.

Methods: This was an open-label, randomized, 3-part study conducted in 127 healthy male (13%) and female (87%) subjects. Plasma concentrations of abemaciclib and its major metabolites were assessed up to 192 hours postdose using validated LC-MS/MS methods.

Part A was a 2-period, crossover pilot study to compare abemaciclib capsules (3 x 50-mg) and tablets (1 x 150-mg) formulations in the fasted state. Results from Part A were used to ensure adequate PK sampling and estimate intra-individual variability to confirm the sample size required for Part B of the study.

Part B was a 3-period, crossover, bioequivalence study of a 50-mg capsule (3 x 50-mg) compared to a 150-mg tablet (1 x 150-mg) and a 50mg tablet (3 x 50-mg) in the fasted state.

Part C was a 2-period crossover food effect study to determine the effect of a high-fat, high-calorie meal on the PK of abemaciclib when administered as a single 150-mg tablet compared to the fasted state.

Abemaciclib $C_{\text{max}}$ and AUC determined using noncompartmental methods were log-transformed and analyzed using a linear mixed-effects model. Geometric least squares (LS) mean for each treatment, the ratio of the geometric LS means, and their 90% CIs were calculated.

Results: In Part A, the abemaciclib intra-subject variability for $C_{\text{max}}$ was 26.7%, requiring a sample size of 76 subjects for Part B to ensure approximately 90% probability that the 90% CIs of the geometric means would be contained within the 0.80 to 1.25 limits, assuming that the true ratio was 1.09.

In Part B, both the 3 x 50-mg and 1 x 150-mg tablets were bioequivalent to the 3 x 50-mg capsules; the 90% CIs of the ratios of geometric LS means for $C_{\text{max}}$ and AUC were contained within the bioequivalence interval of 0.80 to 1.25.

<table>
<thead>
<tr>
<th>Ratio of geometric LS mean (90% CI) compared to 3x50 mg capsule</th>
</tr>
</thead>
<tbody>
<tr>
<td>1x150 mg tablet</td>
</tr>
<tr>
<td>AUC(0-tlast)</td>
</tr>
<tr>
<td>AUC(0-∞)</td>
</tr>
<tr>
<td>$C_{\text{max}}$</td>
</tr>
</tbody>
</table>

In Part C, consumption of a high-fat, high-calorie meal before abemaciclib dosing resulted in an increase of 13% (90% CI 1.05,1.22) in AUC(0-∞) and 30% (90% CI 1.20,1.40) in $C_{\text{max}}$, which was not considered clinically relevant given the magnitude of the effect relative to the inter-subject variability for $C_{\text{max}}$ (36.5%). Also, the magnitude of the change was within the therapeutic window for abemaciclib. There was no difference in median $t_{\text{max}}$ in the fed state compared to the fasted state for abemaciclib. Single 150-mg doses of abemaciclib were generally well tolerated when administered with or without food. The majority of the AEs reported during the study were gastrointestinal and mild in severity.

Conclusions: Abemaciclib 150-mg and 50-mg tablets are bioequivalent to the 50-mg capsule formulation, based on a single dose in the fasted state, and could be administered with or without food.
Title: Development of humanized anti-CD47 monoclonal antibodies with differentiated functional profiles

Robert W Karr, Katherine Liu, Ronald Hiebsch, Benjamin Capoccia, Michael Donio, Kathleen Crowley, Robyn Puro, Sanjay Chanda and Pamela Manning. Tioma Therapeutics, Inc., St. Louis, MO.

Body: CD47 is a cell surface transmembrane protein that binds to signal regulatory protein alpha (SIRPα) on macrophages and results in a “don't eat me” signal that inhibits phagocytosis. Breast cancer cells, both primary and metastatic, frequently overexpress CD47 and exploit this pathway to evade macrophage-mediated destruction. Anti-CD47 monoclonal antibodies (mAbs) block the CD47/SIRPα interaction and promote tumor cell destruction via phagocytosis. Anti-CD47 mAbs also contribute to an anti-tumor T-cell response in immune-competent mice. Therefore, anti-CD47 antibodies represent a new class of immune checkpoint inhibitors that modulate both the innate and adaptive immune systems.

CD47 is expressed on multiple cell types, including tumor cells and normal cells. Many anti-CD47 mAbs block the CD47/SIRPα interaction and cause phagocytosis of tumor cells, but do not directly induce the death of human tumor cells. Tioma has created new humanized anti-CD47 mAbs with novel and differentiated functional profiles to enhance functional heterogeneity. Ti-104, Ti-176 and Ti-108 block the binding of SIRPα to CD47 and increase phagocytosis of human tumor cells. They also induce cell death of human hematological and solid tumor cell lines (including breast cancer lines) in a cell autonomous manner. Cell death was determined by an increase in phosphatidylserine-positive/7AAD-positive tumor cells assessed by flow cytometry following incubation in media containing anti-CD47 mAb or a negative control immunoglobulin. In vitro, these mAbs bind to human tumor cell lines with apparent binding affinities ranging from low pM to low nM, depending on the cell line and method of analysis (solid-phase or cell-based ELISA, flow cytometry or surface plasmon resonance). In vitro, Ti-104 and Ti-108 bind to human RBCs, whereas Ti-176 has markedly reduced binding to human and cynomolgus monkey RBCs.

In a four-week (once-weekly dosing) exploratory safety study in cynomolgus monkeys with Ti-176 and Ti-108, no dose-limiting toxicity or gross pathological or microscopic findings were identified after an initial dose of 5 mg/kg (Week 1) followed by doses of 50 mg/kg (Weeks 2, 3, and 4). Ti-176 treatment resulted in minimal decrease in red cell mass, hemoglobin and hematocrit, which corresponded in vitro to markedly reduced binding to cynomolgus monkey RBCs. Ti-108 caused transient reduction of RBC parameters comparable to some previously reported anti-CD47 mAbs.

Ti-104, Ti-176 and Ti-108 showed potent, dose-dependent efficacy in multiple mouse tumor models, including in the MDA-MB-231 triple-negative breast cancer orthotopic model.

These data provide the preclinical rationale for further evaluation of Ti-104, Ti-176 and Ti-108 as breast cancer treatments.
**Title:** *In vitro* delivery of model fluorescent dyes through the mammary papilla

Samantha L Kurtz¹ and Louise B Lawson¹. ¹Tulane University, New Orleans, LA.

**Body:** In the United States, 100,000 women each year are diagnosed with atypical hyperplasia (AH), significantly increasing their risk of developing breast cancer. However, only 40% of patients accept breast cancer preventive treatment, in part, due to systemic side effects. The localization of pre-cancerous cells within the ducts or lobules of the breast permits a local treatment strategy employing the innate mammary ducts. This expansive tree-like structure extends throughout the entire breast and may serve as a conduit for local delivery of therapeutics, thereby minimizing side effects associated with current preventive treatment strategies. This study examines how lipophilicity influences diffusion through porcine tissue using a model hydrophilic dye, Sulforhodamine B (SRB), and a model lipophilic dye, Nile Red (NR). Porcine nipple or skin tissue was positioned in a Franz diffusion cell and the formulations were applied topically. With equivalent dye concentrations applied to the donor surface, we noted no significant difference in total dye permeation when comparing diffusion through skin versus nipple tissue or when comparing SRB versus NR after 48 hours. However, the diffusion coefficient for both NR and SRB through the nipple was almost 700-fold greater compared to through the skin indicating improved drug delivery via the nipple. Additionally, after 48 hours there was significantly higher retention of SRB in the nipple and skin compared to NR. When we further looked at the distribution of each dye within the nipple using fluorescence microscopy, we saw a distinction in the localization of each dye. Fluorescence micrographs show that lipophilic NR is retained within the ducts while the hydrophilic SRB diffuses throughout the entire tissue section, suggesting that hydrophobicity impacts the ability of molecules to diffuse from the duct into the surrounding tissue. This suggests that lipophilic carriers may be effective for delivery of cancer therapeutics or preventative agents targeting the mammary ducts, where AH is localized.
Title: Effectiveness, safety and quality of life (QoL) results from the German multicenter AVANTI study of 1st-line bevacizumab (BEV)-containing therapy in >2000 patients (pts) with advanced breast cancer (aBC)

Body: Background: In Europe, BEV is approved with either paclitaxel (PAC) or capecitabine (CAP) as 1st-line therapy for HER2-negative aBC. These regimens are being evaluated in routine oncology practice in the German AVANTI (ML22452) observational study.

Methods: Eligible pts had received no prior chemotherapy (CT) for aBC and had no BEV contraindications. CT schedule, diagnostics and frequency of follow-up are at the physician's discretion. Data are collected for 1 y after starting BEV, with 6-monthly follow-up for 1.5 y thereafter. QoL is assessed using EORTC QLQ-C30. Data cutoff for the 3rd interim analysis was Dec 1, 2016.

Results: Between Oct 2009 and Feb 2015, 2056 eligible pts at >300 centers began treatment with BEV+PAC (n=1658) or BEV+CAP (n=398). Median follow-up was 12.7 (range <0.1–50.9) mo. Median treatment duration was 4.4 (95% CI 4.2–4.6) mo for CT and 6.0 (95% CI 5.8–6.5) mo for BEV. Table 1 summarizes PFS. In the overall population, grade 3/4 AEs were reported in 20% of pts (20% BEV+PAC; 22% BEV+CAP) and led to treatment discontinuation in 5% (5% and 5%, respectively). Mean QLQ-C30 scores were relatively stable over time both overall and in subgroups aged <65 vs ≥65 y, indicating maintained QoL during therapy; no relevant QoL differences between age groups were seen (Table 2). To characterize 'long responders', we identified 459 pts with PFS ≥15 mo (410 BEV+PAC; 49 BEV+CAP). Of these, 33% were aged ≥65 y, 15% had triple-negative aBC (TNBC) and 25% had ≥3 metastatic sites. Median treatment duration was 5.1 mo for CT and 10.8 mo for BEV.

Table 1. PFS by subgroup

<table>
<thead>
<tr>
<th>Pts</th>
<th>No. of events/pts (%)</th>
<th>Median PFS (95% CI), mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>1121/2042 (55)</td>
<td>14.2 (13.5–15.3)</td>
</tr>
<tr>
<td>BEV+PAC</td>
<td>902/1646 (55)</td>
<td>15.2 (14.0–16.2)</td>
</tr>
<tr>
<td>BEV+CAP</td>
<td>219/396 (55)</td>
<td>10.9 (10.1–13.4)</td>
</tr>
<tr>
<td>TNBC</td>
<td>187/429 (44)</td>
<td>12.6 (10.7–14.3)</td>
</tr>
<tr>
<td>Non-TNBC</td>
<td>677/1486 (46)</td>
<td>14.7 (13.6–16.1)</td>
</tr>
<tr>
<td>&lt;65 y</td>
<td>577/1325 (44)</td>
<td>15.1 (13.6–16.2)</td>
</tr>
<tr>
<td>≥65 y</td>
<td>344/717 (48)</td>
<td>13.5 (12.5–14.9)</td>
</tr>
<tr>
<td>&lt;3 metastatic sites</td>
<td>678/1522 (45)</td>
<td>14.4 (13.6–15.8)</td>
</tr>
<tr>
<td>≥3 metastatic sites</td>
<td>254/534 (48)</td>
<td>13.5 (11.7–15.5)</td>
</tr>
</tbody>
</table>
Table 2. Mean QoL scores over time, selected scales

<table>
<thead>
<tr>
<th>Scale</th>
<th>Timepoint</th>
<th>All pts</th>
<th>&lt;65 y</th>
<th>≥65 y</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean score</td>
<td>n</td>
<td>Mean score</td>
</tr>
<tr>
<td>Global health status</td>
<td>BL</td>
<td>840</td>
<td>46.5</td>
<td>557</td>
</tr>
<tr>
<td></td>
<td>Wk 9</td>
<td>867</td>
<td>44.0</td>
<td>570</td>
</tr>
<tr>
<td></td>
<td>Wk 33</td>
<td>520</td>
<td>43.3</td>
<td>355</td>
</tr>
<tr>
<td></td>
<td>Wk 54</td>
<td>342</td>
<td>44.6</td>
<td>228</td>
</tr>
<tr>
<td>Physical functioning</td>
<td>BL</td>
<td>836</td>
<td>68.5</td>
<td>555</td>
</tr>
<tr>
<td></td>
<td>Wk 9</td>
<td>863</td>
<td>60.9</td>
<td>568</td>
</tr>
<tr>
<td></td>
<td>Wk 33</td>
<td>513</td>
<td>60.3</td>
<td>352</td>
</tr>
<tr>
<td></td>
<td>Wk 54</td>
<td>341</td>
<td>61.6</td>
<td>228</td>
</tr>
<tr>
<td>Social functioning</td>
<td>BL</td>
<td>827</td>
<td>61.4</td>
<td>555</td>
</tr>
<tr>
<td></td>
<td>Wk 9</td>
<td>856</td>
<td>54.4</td>
<td>566</td>
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<td>Wk 33</td>
<td>514</td>
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<td>351</td>
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<td></td>
<td>Wk 54</td>
<td>337</td>
<td>58.1</td>
<td>225</td>
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<tr>
<td>Fatigue</td>
<td>BL</td>
<td>835</td>
<td>48.9</td>
<td>555</td>
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<td></td>
<td>Wk 9</td>
<td>860</td>
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<td>567</td>
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<td>56.1</td>
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<td></td>
<td>Wk 54</td>
<td>340</td>
<td>55.8</td>
<td>228</td>
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<tr>
<td>Pain</td>
<td>BL</td>
<td>835</td>
<td>37.8</td>
<td>556</td>
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<tr>
<td></td>
<td>Wk 9</td>
<td>864</td>
<td>38.5</td>
<td>569</td>
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<tr>
<td></td>
<td>Wk 33</td>
<td>517</td>
<td>46.9</td>
<td>353</td>
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<tr>
<td></td>
<td>Wk 54</td>
<td>340</td>
<td>46.9</td>
<td>228</td>
</tr>
</tbody>
</table>

BL=baseline.  aHigher score=better QoL/functioning.  bHigher score=greater symptom burden.

Conclusions: More mature results from AVANTI show median PFS of 14.2 mo, favorable tolerability and maintained QoL, supporting 1st-line use of BEV for aBC. The subgroup with prolonged disease control (PFS >15 mo) was heterogeneous; further analyses of these pts are ongoing.
Title: Clinical efficacy of a humanized monoclonal antibody against the Lewis-Y antigen (Le\(^y\)): Results of a phase II clinical trial

Laura Testa\(^1,2\), Sergio V Serrano\(^3\), Roberto J Arai\(^1\), Marina C Zorzetto\(^3\), Ruffo Freitas-Junior\(^4\), Oren Maletz\(^5\), Max S Mano\(^1\) and Paulo M Hoff\(^1,2\). \(^1\)Instituto do Cancer do Estado de Sao Paulo, Sao Paulo, SP, Brazil; \(^2\)Hospital Sirio Libanes, Sao Paulo, SP, Brazil; \(^3\)Hospital de Câncer de Barretos - Fundação Pio XII, Barretos, SP, Brazil; \(^4\)Universidade Federal de Goiás, Goiania, GO, Brazil and \(^5\)Recepta Biopharma.

Body: Background. The Lewis-Y (Le\(^y\)) antigen is a blood group-related antigen expressed in over 70% of epithelial cancers. It is expressed in 44% of breast cancers.

Objectives. The primary endpoint was to evaluate the clinical efficacy of hu3S193, a humanized monoclonal antibody against the Lewis-Y antigen, in advanced hormone positive breast cancer after failure of at least one line of endocrine therapy.

Methods. This multicenter, single arm, phase II trial enrolled eligible patients to receive hu3S193 weekly at a dose of 20 mg/m\(^2\) intravenously. Efficacy was measured as clinical benefit rate (objective response or stable disease for at least 24 weeks).

Results. Of 49 patients screened, 27 (55%) were Le\(^y\) positive. Of these 27, only 20 were eligible for efficacy analysis. No complete or partial responses were observed. Five patients had stable disease for 24+ weeks (clinical benefit rate 25%). One patient remains on study drug maintaining stable disease for over 2 years. This patient had high expression of Le\(^y\). The most common treatment-related adverse events were headache (50%), cough (45.5%) and nausea/vomiting (31.8%).

Conclusions. Hu3S193 lacked sufficient activity in is trial and the investigators stopped accrual at the first interim analysis. High expression of Le\(^y\) might play a role in selecting patients to this strategy.
Title: Duration of adjuvant trastuzumab in HER-2 positive breast cancer: Pooled results of overall, and disease-free survivals from meta-analyses of randomized controlled trials

Saroj Niraula¹ and Bishal Gyawali². ¹University of Manitoba and CancerCare Manitoba, Winnipeg, MB, Canada and ²Institute of Cancer Policy, London, United Kingdom.

Body: Background: The duration of one year of trastuzumab for treatment of HER-2 positive breast cancer was chosen arbitrarily. Randomized controlled trials (RCTs) have since explored efficacy of shorter durations of trastuzumab compared to 1 year, but the results from such RCTs are inconsistent, and are subject to different interpretations due mainly to varying definitions of non-inferiority.

Methods: Systematic search of pubmed, embase, and major conference proceedings was performed to identify RCTs comparing outcomes of one year versus shorter trastuzumab in the adjuvant treatment of breast cancer. Efficacy outcomes [Hazard Ratios (HR) for Overall Survival (OS) and Disease Free Survival (DFS) with respective 95% Confidence Intervals (CI)] and toxicity outcome [Odds Ratios (OR) and 95% CI for cardiac events] from each study were weighted using generic inverse variance approach and pooled in a meta-analysis using RevMan 5.3 software. Heterogeneity among studies was assessed using tau² and i² statistics. Interactions on overall results by hormone receptor status, and nodal status were assessed using chi² statistics.

Results: Three RCTs involving 5,114 patients reported outcomes on both OS and DFS. Two studies evaluated 6 months and 1 study evaluated 9 weeks of adjuvant trastuzumab, compared to 1 year. Individual RCTs had failed to demonstrate non-inferiority of shorter duration compared to 1 year based on various pre-specified upper limits of CI (of <1.15, <1.29 and <1.59, respectively). Pooled analyses demonstrated statistically significant improvements in both OS (HR 0.78, 95% CI: 0.62-0.98), and DFS (HR 0.80, 95% CI 0.73-0.93) with the use of 1 year of trastuzumab compared to shorter durations suggesting superiority favoring 1 year of treatment, despite non-inferiority designs of individual trials. Sensitivity analyses based on duration of the experimental arm (9 weeks or 6 months) did not influence the direction, or size of overall effects. Pooled sub-group analyses demonstrated no interaction by hormone receptor status, or nodal status on overall results (p for sub-group difference = 0.73, and 0.52, respectively). Odds of cardiac events increased significantly with 1 year of treatment. (table 1)

Conclusion: Pooled analyses of RCTs demonstrated a significant improvement in overall, and disease-free survivals with 1 year of adjuvant trastuzumab compared to shorter durations for adjuvant treatment of HER-2 positive breast cancer. One year of trastuzumab should remain as the standard of care. Cardiotoxicity increased significantly with the longer treatment.

<table>
<thead>
<tr>
<th>OUTCOMES</th>
<th>HAZARD RATIO</th>
<th>95% CONFIDENCE INTERVAL</th>
<th>p (DIFFERENCE)</th>
<th>INTERACTION TEST</th>
</tr>
</thead>
<tbody>
<tr>
<td>OVERALL SURVIVAL</td>
<td>0.78</td>
<td>0.62 - 0.98</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>DISEASE FREE SURVIVAL (DFS)</td>
<td>0.80</td>
<td>0.70 - 0.93</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>DFS - ESTROGEN RECEPTOR POSITIVE</td>
<td>0.82</td>
<td>0.66 - 1.03</td>
<td>0.09</td>
<td>CHI SQ. 0.12; p(SUBGROUP-DIFFERENCE)0.73</td>
</tr>
<tr>
<td>DFS - ESTROGEN RECEPTOR NEGATIVE</td>
<td>0.78</td>
<td>0.63 - 0.97</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>DFS - NODE POSITIVE</td>
<td>0.76</td>
<td>0.63 - 0.92</td>
<td>0.004</td>
<td>CHI SQ. 0.42; p(SUBGROUP-DIFFERENCE)0.52</td>
</tr>
<tr>
<td>DFS - NODE NEGATIVE</td>
<td>0.87</td>
<td>0.60 - 1.26</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td>CARDIAC EVENTS</td>
<td>2.65 (ODDS)</td>
<td>2.00 - 3.50</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>
**Title:** Effects of neratinib after trastuzumab-based adjuvant therapy in hormone receptor-positive HER2+ early-stage breast cancer: Exploratory analyses from the phase III ExteNET trial

Stephen KL Chia¹, Miguel Martin², Hiroji Iwata³, Beverly Moy⁴, Alshad S Lalani⁵, Frankie Ann Holmes⁶, Janine Mansi⁷, Gunter von Minckwitz⁸, Marc Buyse⁹, Suzette Delaloge¹⁰, Bent Ejertsen¹¹, Bin Yao⁵, Adolfo Murias Rosales¹², Beth Hellerstedt¹³, Søren Cold¹⁴, Kenichi Inoue¹⁵, Zhen-Zhou Shen¹⁶, Teresa Galeano¹⁷, Carlos H Barrios¹⁸ and Arlene Chan¹⁹.¹ British Columbia Cancer Agency, Vancouver, BC, Canada; ²Hospital General Universitario Gregorio Marañón; ³Aichi Cancer Center Hospital; ⁴Massachusetts General Hospital Cancer Center; ⁵Puma Biotechnology Inc.; ⁶Texas Oncology; ⁷Guy’s and St Thomas’ NHS Foundation Trust and Biomedical Research Centre, King’s College London; ⁸German Breast Group; ⁹International Drug Development Institute; ¹⁰Institut Gustave Roussy; ¹¹Rigshospitalet; ¹²Compejo Hospitalario Materno Insular de Las Palmas; ¹³Texas Oncology, P.A; ¹⁴Odense University Hospital; ¹⁵Saitama Cancer Center; ¹⁶Shanghai Cancer Center; ¹⁷Magna Graecia University; ¹⁸Pontifical Catholic University of Rio Grande do Sul School of Medicine and ¹⁹Breast Cancer Research Centre-Western Australia and Curtin University.

**Body:** Background: The international, randomized, placebo-controlled phase III ExteNET trial showed that a 1-year course of neratinib after trastuzumab-based adjuvant therapy significantly improved 2-year invasive disease-free survival (iDFS) in patients with early-stage HER2+ breast cancer (BC) (hazard ratio 0.67; 95% CI 0.50–0.91; p=0.009) [Chan et al. Lancet Oncol 2016]. The significant iDFS benefit with neratinib was maintained after a median 5 years' follow-up (hazard ratio 0.73; 95% CI 0.57-0.92; p=0.008) [Martin et al. ESMO 2017]. At both time-points, marked benefit with neratinib was evident in patients with hormone receptor (HR)+ tumors, whereas in patients with HR– disease, initial improvements with neratinib diminished after completing treatment. We report exploratory analyses from the ExteNET trial done to better characterize the effects of neratinib in the HR+ subgroup.

**Methods:** Patients with early-stage HER2+ BC were randomly assigned to oral neratinib 240 mg/day or placebo for 1 year after standard primary therapy and trastuzumab-based adjuvant therapy. Randomization was stratified by HR status (locally assessed), nodal status, and trastuzumab regimen. Adjuvant endocrine therapy was recommended for patients with HR+ disease. Data concerning disease recurrences were collected prospectively during year 1-2 post-randomization, and from medical records during year 3–5 post-randomization. Primary endpoint: iDFS. Secondary endpoints: DFS including ductal carcinoma in situ (DFS-DCIS); time to distant recurrence (TTDR); distant DFS (DDFS); cumulative incidence of central nervous system (CNS) recurrences; overall survival (OS). Hazard ratios (95% CI) were estimated using Cox proportional-hazards models. Data cut-off: March 2017. Clinicaltrials.gov: NCT00878709.

**Results:** 2840 patients were randomized (neratinib, n=1420; placebo, n=1420); 1631 (57%) patients had HR+ tumors (neratinib, n=816; placebo, n=815). 93% and 94% of HR+ patients in the neratinib and placebo groups, respectively, were receiving adjuvant endocrine therapy at baseline. Efficacy outcomes in the HR+ cohort after a median follow-up of 5.2 years are shown in the table. In subgroup analyses of the HR+ cohort, hazard ratios for iDFS were 0.49 in centrally confirmed HER2+ patients (n=951), and 0.58 in patients who had completed prior trastuzumab ≤12 months before randomization (n=1334). CNS recurrence and OS data are not yet mature.

<table>
<thead>
<tr>
<th></th>
<th>Updated 2-year analysis</th>
<th>5-year analysis</th>
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<tbody>
<tr>
<td></td>
<td>Hazard ratio</td>
<td>P-value</td>
</tr>
<tr>
<td></td>
<td>(95% CI)</td>
<td>(2 sided)</td>
</tr>
<tr>
<td>iDFS</td>
<td>4.1</td>
<td>0.49 (0.31–0.75)</td>
</tr>
<tr>
<td>DFS-DCIS</td>
<td>4.8</td>
<td>0.45 (0.29–0.69)</td>
</tr>
<tr>
<td>DDFS</td>
<td>3.1</td>
<td>0.52 (0.32–0.84)</td>
</tr>
<tr>
<td>TTDR</td>
<td>2.9</td>
<td>0.52 (0.31–0.85)</td>
</tr>
</tbody>
</table>
Conclusions: Neratinib was associated with an absolute iDFS benefit of 4.4% in patients with HR+/HER2+ BC after 5 years’ follow-up. HR/HER2 receptor cross-talk may underpin the notable effect of neratinib in patients with HR+ tumors when given in combination with endocrine therapy.
Impact of hormone receptor status in HER2-Positive early breast cancer in the trastuzumab era: Results of a National multi-institutional study

Alexandre de Nonneville, Anthony Gonçalves, Monique Cohen, Fabien Reyal, Jean Marc Classe, Sylvia Giard, Pierre Emmanuel Colombo, Xavier Muracciole, Nicolas Chopin, Eric Lambaudie and Gilles Houvenaeghel. 1Aix-Marseille Univ, CNRS, INSERM, Institut Paoli-Calmettes, CRCM, Marseille, France; 2Aix-Marseille Univ, CNRS, INSERM, Institut Paoli-Calmettes, CRCM, Marseille, France; 3Institut Curie, Paris, France; 4Institut René Gauducheau, St Herblain, France; 5Centre Oscar Lambret, Lille, France; 6CRLC Val-d’Aurelle, Montpellier, France; 7Hôpital de la Timone, Marseille, France and 8Centre Léon Bérard, Lyon, France.

Body: Background: Recent updated analysis of the HERA (HERceptin Adjuvant) trial indicate that tumor hormone receptor status (HR) remains a major determinant of outcome in HER2-positive (HER2+) early breast cancer (BC) patients, with higher rates of recurrence and death in women with HR-negative (HR-) disease, even after 11 years’ median follow-up. Furthermore, data reported from the HERA trial suggest that the timing of recurrences is different, with an initial higher frequency of disease-free survival (DFS) events in patients with HR- disease than those with HR-positive disease (HR+). No evidence of a different trastuzumab efficacy according to the HR of the primary tumor was found. In this study, we examined the impact of HR on outcome in a large, multicenter, “real-world”, retrospective cohort of HER2+ early breast cancer patients.

Methods: HER2+ BC were retrospectively identified from a large cohort of 23,375 consecutive patients who underwent primary surgery at 17 French centers between Dec 1987 and Jan 2014. A multivariate Cox model was built including age, tumor size, SBR grade, lymphovascular invasion, lymph node involvement, hormonal receptors status, adjuvant chemotherapy, adjuvant hormone therapy, trastuzumab, radiotherapy and type of surgery.

Results: A total of 1308 cases were identified, including 829 (63%) HR+ and 479 (47%) HR- patients. Median follow-up was 52 months (range 0 to 201). Compared with HR+, HR- patients had significantly smaller tumors (37 vs. 31% ≤ 10mm, p=0.027; information for multifocal tumors was not available), with higher SBR grade (58 vs. 40% grade 3, p<0.001) and had more lymph nodes involvement (41 vs. 32% pN+, p=0.001). HR- patients were more frequently treated by mastectomy (41 vs. 31%, p<0.001), received more trastuzumab (63 vs. 53%, p<0.001) and less radiotherapy (85 vs. 89%, p=0.020). Endocrine therapy was administered in 90% (744) of HR+ patients. No other significant difference in patient, tumor or treatment characteristics was found. HR status impacted DFS, metastasis free-survival (MFS) and BC-Specific survival (BC-SS) (hazard ratios: 0.46 [0.32-0.66]; p<0.001, 0.52 [0.33-0.82]; p=0.004 and 0.56 [0.34-0.90]; p=0.017, respectively, log-rank test) in overall population with higher rates of recurrence and death in women with HR- disease. In multivariate analysis, lymph node involvement and use of trastuzumab but not HR status impacted significantly DFS, MFS and BC-SS. Considering patients by treatment groups (with or without trastuzumab), HR status was not predictive of survival outcomes in the trastuzumab group, as opposed to the group without trastuzumab. Regarding the timing of recurrences, we observed an increased tendency for later relapse in patients with HR+ disease compared with HR- disease, for both DFS and MFS events.

Conclusions: Our results suggest that HR status remains a major determinant of outcome in HER2+ BC, including the timing of recurrence. Yet, this prognostic impact appears to be mitigated by trastuzumab-based adjuvant treatment.
2017 San Antonio Breast Cancer Symposium

Publication Number: P1-13-05

Title: Timing of initiation of neratinib after completion of trastuzumab-based adjuvant therapy in early-stage HER2+ breast cancer: Exploratory analyses from the phase III ExteNET trial


1Rigshospitalet, Copenhagen, Denmark; 2Breast Cancer Research Centre-Western Australia and Curtin University; 3Comprehensive Cancer Centre, Medical University of Vienna; 4German Breast Group; 5Institut Gustave Roussy; 6International Drug Development Institute; 7Texas Oncology-Baylor Charles A. Sammons Cancer Center; 8Guy's and St Thomas' NHS Foundation Trust and Biomedical Research Centre, King's College London; 9Massachusetts General Hospital Cancer Center; 10Aichi Cancer Center Hospital; 11Puma Biotechnology Inc; 12Vanderbilt-Ingram Cancer Center; 13Westmead Hospital and the University of Sydney, Sydney, NSW, Australia; 14Hospital Universitario Virgen del Rocio; 15Instituto Valenciano de Oncología; 16Shanghai Cancer Center; 17Texas Oncology; 18Szpital Morski im. PCK Oddzial Onkologii Klinicznej, Gdyńskie Centrum Onkologii and 19Hospital General Universitario Gregorio Marañón.

Body: Background: The international, randomized, placebo-controlled phase III ExteNET trial showed that 1 year of neratinib after trastuzumab-based adjuvant therapy significantly improved 2-year invasive disease-free survival (iDFS) in early-stage HER2+ breast cancer (HR 0.67; 95% CI 0.50–0.91; p=0.009) [Chan et al. Lancet Oncol 2016]. The significant iDFS benefit with neratinib was maintained after a median of 5 years' follow-up (HR 0.73; 95% CI 0.57-0.92; p=0.008) [Martin et al. ESMO 2017]. We present exploratory analyses from the ExteNET trial examining the effects of the interval between completion of trastuzumab and randomization to commence neratinib on iDFS.

Methods: Women with early-stage HER2+ breast cancer were randomly assigned to oral neratinib 240 mg/day or placebo for 1 year after standard primary therapy and trastuzumab-based adjuvant therapy. Under the original study protocol, (neo)adjuvant trastuzumab was to be completed ≤24 months before randomization; this was revised to ≤12 months before randomization after the NCCTG-N9831/NSABP B-31 4-year analysis showed that the risk of relapse is greatest during the first 12 months after completing trastuzumab. Disease recurrences were collected prospectively during 1 and 2 years post-randomization, and from medical records during 3–5 years post-randomization. Patients randomized ≤12 months after completion of adjuvant trastuzumab were further separated to look at those who initiated neratinib ≤6 months of completing adjuvant trastuzumab. Primary endpoint: iDFS. HR (95% CI) estimated using Cox proportional-hazards models. Data cut-off: March 1, 2017. Clinicaltrials.gov: NCT00878709.

Results: The intention-to-treat population comprised 2840 patients (neratinib, n=1420; placebo, n=1420). Median time from last trastuzumab dose to randomization was 4.4 and 4.6 months in the neratinib and placebo groups, respectively. 81% of patients were randomized ≤12 months of completing trastuzumab. The effects of the interval between the last dose of trastuzumab and randomization/initiation of neratinib on iDFS after a median follow-up of 5.2 years are shown in the table.

<table>
<thead>
<tr>
<th>Interval from last dose of trastuzumab to randomization</th>
<th>n</th>
<th>Neratinib</th>
<th>Placebo</th>
<th>HR (95% CI) a</th>
<th>P-value (2-sided)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤6 months</td>
<td>1641</td>
<td>90.0</td>
<td>85.4</td>
<td>0.62 (0.46–0.84)</td>
<td>0.002</td>
</tr>
<tr>
<td>≤12 months b</td>
<td>2297</td>
<td>89.7</td>
<td>86.5</td>
<td>0.70 (0.54–0.90)</td>
<td>0.006</td>
</tr>
<tr>
<td>&gt;12 months b</td>
<td>543</td>
<td>92.3</td>
<td>92.6</td>
<td>1.00 (0.51–1.94)</td>
<td>0.992</td>
</tr>
</tbody>
</table>

a. Neratinib vs placebo; b. Protocol-defined subgroups

Conclusions: In ExteNET, patients who initiated neratinib within 12 months of completing trastuzumab-based adjuvant therapy...
appeared to derive greater benefit from treatment than those who started neratinib later. Further, exploratory analyses suggest that the magnitude of benefit with neratinib is greater if initiated sooner (i.e. within 6 months of completing trastuzumab). Given the benefits of neratinib overall in those initiating treatment ≤12 months from the end of adjuvant trastuzumab, extended adjuvant treatment with neratinib should be initiated early following completion of trastuzumab.
Title: Safety of trastuzumab in the subcutaneous formulation for the treatment of HER2 positive early breast cancer (eBC) patients: Primary results of SCHEARLY trial

Milvia Zambetti¹, Filippo Montemurro², Paolo Morandi³, Claudio Zamagni⁴, Alessia Stell⁵, Antonella Rozzi⁶ and Luca Gianni¹.
¹Ospedale San Raffaele, Milano, Italy; ²Istituto di Candiolo IRCCS, Candiolo, Italy; ³Ospedale dell'Angelo, Venezia, Italy; ⁴Policlinico S.Orsola-Malpighi, Bologna, Italy and ⁵Roche SpA, Monza, Italy.

Body:
Background: This study in Italian breast cancer patients is part of a phase IIIB, open-label, multinational umbrella study (UmbHER1), assessing the safety and tolerability of the subcutaneous (SC) formulation of Trastuzumab as primary end point. Present analysis is relative to the safety during the treatment period, with a major focus to side effects associated with the Trastuzumab SC administration.

Methods: Patients with HER2-positive eBC and LABC were eligible and included in two sequential cohorts: 120 patients treated with Trastuzumab SC by handheld syringe (Cohort A), and 120 with Trastuzumab SC by single-use injection device (SID, Cohort B). All the patients received adjuvant or neo-adjuvant treatment (clinician choice) with Anthracycline-containing regimens (FEC/EC/AC) followed by Trastuzumab SC in combination with taxanes (weekly Paclitaxel or Docetaxel) and then in monotherapy for a total of 18 cycles. Safety clinical and instrumental evaluations were planned at definite time points.

Results: 240 patients were enrolled and 202 patients (82.6% in cohort A and 84.9% in cohort B) completed the treatment. Reasons for discontinuation were the following: 7.9% AE/intercurrent illness, 3.3% withdrew consent, 1.7% recurrence of disease, 1.7% refused treatment, 0.9% violation of inclusion criteria, 0.9% other reasons. In the safety population, 98.2% of patients experienced at least one adverse event from the start of the Anthracycline treatment until the Safety Follow-up visit. Patients experiencing a Treatment Emergent (from Trastuzumab start date) AEs defined as Grade ≥3 were 26.8%; of these, 3.9% were considered related to study drug; Treatment Emergent Serious Adverse Events appeared in 7.5% of the safety population, of which 0.9% were considered related to study drug: one pleuropericarditis and one anaphylactic shock, both resolved. The frequency of systemic administration-related reactions, ARRs (pyrexia 25%, erythema 20.2%, rash 7.0%, chest pain 7.0%, pruritus 6.6%, chills 1.3%, anaphylactic shock 0.9%) and local injection site reactions, ISRs (pain 6.6%, injection site reactions 3.9%, subcutaneous abscess 0.9%, administration site oedema 0.4%) potentially related to the subcutaneous formulation is cumulatively reported below.

<table>
<thead>
<tr>
<th></th>
<th>% all / G≥3</th>
<th>% related to IMP</th>
<th>% related cohort A</th>
<th>% related cohort B</th>
<th>% during Tax+Trast all/ G≥3</th>
<th>% during Trast only all/ G≥3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic ARRs</td>
<td>68.0 / 2.2</td>
<td>8.8</td>
<td>5.3</td>
<td>3.9</td>
<td>16.7 / 0.4</td>
<td>25.0 / 0.4</td>
</tr>
<tr>
<td>Local ISRs</td>
<td>21.9 / 0.9</td>
<td>7.0</td>
<td>4.8</td>
<td>1.3</td>
<td>6.6 / 0.4</td>
<td>3.1 / 0.0</td>
</tr>
</tbody>
</table>

The mean reduction of Left Ventricular Ejection Fraction (LVEF) at the end of 1 year treatment from screening was 2.9%. 8.8% of patients experienced a decrease in LVEF, in most cases defined related to study drug (18 patients out of 20); no cases of CHF were reported.

Conclusion: Local and systemic tolerability of subcutaneous Trastuzumab administration is good in both groups and there is no evidence of increased incidence and severity of IMP-related systemic side effects in comparison with the standard intravenous route.
**Title:** Incidence and management of diarrhea with adjuvant pertuzumab and trastuzumab in HER2-Positive breast cancer

Jose Bines¹, Evandro de Azambuja², Dimitrios Zardavas³, Marion Procter⁴, Eleonora Restuccia⁵, Giuseppe Viale⁶, Thomas Suter⁷, Amal Arahmani⁸, Veerle van Dooren⁹, Emma Clark¹⁰, Jennifer Eng-Wong¹¹, Richard Gelber¹², Martine Piccart¹³, Gunter von Minckwitz¹⁴ and Jose Baselga¹⁵. ¹Instituto Nacional de Câncer, Rio de Janeiro, Brazil; ²Breast European Adjuvant Study Team (BrEAST) Data Center, Brussels, Belgium; ³Breast International Group (BIG), Brussels, Belgium; ⁴Frontier Science (Scotland), Kincraig, United Kingdom; ⁵Roche Pharma, Basel, Switzerland; ⁶European Institute of Oncology, University of Milan, Milan, Italy; ⁷Bern University Hospital, Bern, Switzerland; ⁸Breast International Group (BIG), Brussels, Belgium; ⁹Breast European Adjuvant Study Team (BrEAST) Data Center, Brussels, Belgium; ¹⁰Roche Pharma, Basel, Basel, Switzerland; ¹¹Genentech, San Francisco; ¹²Dana-Farber Cancer Institute, Harvard Medical School, Harvard T. H. Chan School of Public Health, and Frontier Science and Technology Research Foundation, Boston; ¹³Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium; ¹⁴German Breast Group, Neu-Isenburg, Germany and ¹⁵Memorial Sloan Kettering Cancer Center, New York.

**Body: Background**
Diarrhea is the most commonly reported adverse event (AE) on pertuzumab (Ptz) in both early and metastatic breast cancer (BC) settings. We report safety analyses of diarrhea from the large adjuvant APHINTITY study in HER2 positive early breast cancer (EBC).

**Patients and methods**
In this exploratory analysis, the safety population included 2364 patients in the Ptz arm and 2405 in the placebo (Pla) arm. No specific prophylaxis was mandated by the protocol, however early intervention with loperamide as well as fluid and electrolyte replacement was recommended. Diarrhea incidence, severity (NCI-CTCAE v4.0), onset and management were analyzed.

**Results**
Diarrhea was the most common AE in the Ptz arm (71.3% vs. 45.2% in the Pla arm) and the events were mostly G1. Diarrhea ≥G3 was observed in 9.8% and 3.7% in Ptz and Pla arms, respectively. The highest incidence was reported during administration of HER2 targeted therapy and taxane (61.4% vs. 33.8% with Ptz and Pla, respectively) with a marked decrease observed upon chemotherapy cessation (18.1% vs. 9.2% with Ptz and Pla, respectively). The median time from first targeted treatment to onset of diarrhea during the chemotherapy phase was 7 and 10 days (Ptz/Pla). On average, diarrhea events lasted longer in the Ptz than in the Pla arm (median 8 vs. 6 days). Diarrhea events were more frequent with the administration of docetaxel + carboplatin and targeted agents, irrespective of the severity. Detailed results are reported in Table 1.

**Conclusions**
In the curative setting, diarrhea due to Ptz was mild, generally manageable with common antidiarrheals and did not affect patients’ ability to receive treatment. The APHINTITY findings are consistent with the well-characterized pattern of pertuzumab-related diarrhea across the HER2 BC spectrum.

Diarrhea incidence, severity (NCI-CTCAE v4.0), onset and management

<table>
<thead>
<tr>
<th></th>
<th>Ptz, n=2364</th>
<th>Pla, n=2405</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Incidence and severity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total number of patients with at least one adverse event $</td>
<td>1685 (71.3%)</td>
<td>1086 (45.2%)</td>
</tr>
<tr>
<td>Total number of events $</td>
<td>3415</td>
<td>1792</td>
</tr>
<tr>
<td>NCI CTC AE Grade (highest grade per patient)!</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>1683 (71.2%)</td>
<td>1085 (45.1%)</td>
</tr>
<tr>
<td>Grade 1</td>
<td>829 (35.1%)</td>
<td>690 (28.7%)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>622 (26.3%)</td>
<td>305 (12.7%)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>229 (9.7%)</td>
<td>90 (3.7%)</td>
</tr>
<tr>
<td>Grade 4</td>
<td>3 (0.1%)</td>
<td>0</td>
</tr>
<tr>
<td>Onset and duration$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Median time (days) from 1st HER2 targeted treatment to onset (min-max)</td>
<td>7 (1 – 358)</td>
<td>10 (1 - 384)</td>
</tr>
<tr>
<td>Median Duration (days) of each event (min-max)</td>
<td>8 (1 - 811)</td>
<td>6 (1 - 1022)</td>
</tr>
</tbody>
</table>

**Management**

<table>
<thead>
<tr>
<th>Antidiarrheals$</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>898 (38.0%)</td>
<td>386 (16.0%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dose modification* of any study drug!</th>
<th></th>
</tr>
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<tbody>
<tr>
<td>210 (8.9%)</td>
<td>74 (3.1%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dose modification* of HER2 targeted treatment!</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>69 (2.9%)</td>
<td>18 (0.7%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Discontinuation of any study drug!</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>38 (1.6%)</td>
<td>7 (0.3%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Discontinuation of HER2 Targeted treatment!</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>20 (0.8%)</td>
<td>2 (&lt;0.1%)</td>
</tr>
</tbody>
</table>

$ Based on a basket of preferred terms for diarrhea! Based only on the preferred term diarrhea * Includes dose reductions (chemotherapy only), delays or interruptions during infusion
Title: Extended adjuvant neratinib/fulvestrant blocks ER/HER2 crosstalk and maintains complete responses of ER+/HER2+ tumors following treatment with chemotherapy and anti-HER2 therapy

Dhivya R Sudhan¹, Luis J Schwarz¹, Angel L Guerrero-Zotano¹, Mellissa Nixon¹, Luigi Formisano¹, Sarah Croessmann¹, Paula I Gonzalez Ericsson¹, Melinda E Sanders¹, Justin M Balko¹, Francesca Avogadri-Connors², Richard E Cutler², Alshad S Lalani², Richard Bryce², Alan Auerbach² and Carlos L Arteaga¹. ¹Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center, Nashville, TN and ²Puma Biotechnology Inc., Los Angeles, CA.

Body: Background: Neratinib is a potent, irreversible pan-HER tyrosine kinase inhibitor. The phase III trial ExteNET showed improved disease-free survival in patients (pts) with HER2+ breast cancer treated with neratinib vs placebo after trastuzumab-based adjuvant therapy. The benefit from neratinib appeared to be greater in pts with ER+ tumors. Thus, we sought to elucidate mechanisms that may explain the benefit from extended adjuvant therapy with neratinib in pts with ER+/HER2+ breast cancer using a human-in-mouse model that simulates the clinical outcomes seen in ExteNET.

Results: Mice with established ER+/HER2 amplified MDA-361 tumors were treated with trastuzumab (tz) + paclitaxel (pac) for 4 weeks, and then randomized to fulvestrant (fulv) ± neratinib for 4 weeks. All MDA-361 tumors exhibited a prompt and marked reduction in volume after tz/pac treatment; 10 mice achieved a complete response (CR) before receiving 'extended adjuvant' therapy with fulv (n=5) or neratinib/fulv (n=5). A CR was maintained with neratinib/fulv following tz/pac. However, mice treated with fulv alone, relapsed rapidly (p<0.05 at week 8) despite of a complete downregulation of tumor ER levels. In a second experiment, nude mice with established MDA-361 xenografts were treated with pertuzumab/tz/pac for 4 weeks. Following a CR, mice were randomized to neratinib/fulv vs. fulv. Again, mice treated with neratinib/fulv maintained a CR, while mice in the fulv alone arm exhibited tumor progressions within a week. In three ER+/HER2+ cell lines (MDA-361, BT474 and UACC893) but not in ER+/HER– MCF7 cells, treatment with neratinib induced ER reporter transcriptional activity whereas treatment with fulv resulted in an increase in HER2 phosphorylation, suggesting compensatory crosstalk between the ER and HER2 pathways. To further understand the molecular basis of this crosstalk, MDA-361 tumor-bearing mice were treated with either fulv, neratinib or the combination for 7 days, after which tumors were harvested and analyzed using a Nanostring breast cancer ER panel consisting of 196 ER-regulated genes. Compared to vehicle or fulv-treated tumors, tumors treated with neratinib alone and neratinib/fulv showed marked downregulation of cyclin D1 mRNA expression. Similarly, in MDA-361, BT474 and UACC893 cells but not in MCF7 cells, only neratinib/fulv downregulated cyclin D1, P-AKT and P-ERK. Finally, treatment with neratinib/fulv but not fulv alone reduced cyclin D1 transcriptional reporter activity and cyclin D1 protein levels, and induced cell cycle arrest, suggesting double blockade is required to overcome compensatory crosstalk between ER and amplified HER2.

Conclusions: Neratinib/fulv but not fulv alone maintained complete responses of ER+/HER+ tumors following treatment with tz/pac or pertuzumab/tz/pac, reminiscent of the results in ExteNET. Neratinib treatment promoted ER transcriptional activity whereas ER downregulation with fulv was associated with increased HER2 signaling. In ER+/HER2+ breast cancer cells and tumors, neratinib/fulv synergistically inhibited growth, cyclin D1 expression, and AKT and MAPK activation, thus providing a plausible mechanism to explain the results in the ExteNET trial.
Title: Adjuvant treatment of HER2+ breast cancer: Should trastuzumab be given sequentially or concurrently with chemotherapy?

Gwen MHE Dackus, Katarzyna Jóźwiak, Elsken Van der Wall, Paul J Van Diest, Michael Hauptmann, Sabine Siesling, Gabe S Sonke, and Sabine C Linn.

1. Netherlands Cancer Institute, Amsterdam, Noord-Holland, Netherlands; 2. University Medical Center Utrecht, Utrecht, Netherlands; 3. Netherlands Cancer Institute, Amsterdam, Noord-Holland, Netherlands; 4. University Medical Center Utrecht, Utrecht, Netherlands; 5. Netherlands Comprehensive Cancer Organisation, Utrecht, Netherlands; 6. MIRA Institute for Biomedical Technology and Technical Medicine, University of Twente, Enschede, Overijssel, Netherlands and 7. Netherlands Cancer Institute, Amsterdam, Noord-Holland, Netherlands.

Body: Background

Human Epidermal growth factor Receptor 2 positive (HER2+) breast cancers have a high risk of recurrence in the absence of systemic treatment. The monoclonal antibody trastuzumab in combination with chemotherapy has significantly improved survival. Randomized trials have given trastuzumab both concurrently and sequentially with chemotherapy. To date, only one study reported a comparison between concurrent and sequential trastuzumab, with a numerically but not statistically significant benefit for concurrent use.

Our aim is to evaluate whether there is a difference in survival between patients who received trastuzumab sequentially to chemotherapy compared to concurrently with chemotherapy using data from the population-based, Netherlands Cancer Registry (NCR).

Methods

All women diagnosed in the Netherlands with a HER2+, T_{any}N_{any}M_0 breast tumor between 2005 and 2007 who received both chemotherapy and trastuzumab were identified from the NCR. Kaplan Meier survival estimates and Cox regression were used to compare recurrence free survival (RFS) and overall survival (OS) by trastuzumab sequence. Hazard ratios (HR) were adjusted for grade, pathological T-stage, pathological N-stage, estrogen receptor (ER), progesterone receptor, radiotherapy, hormonal therapy and ovarian ablation.

Results

A total of 1,849 patients were identified, with a mean follow-up of 7.8 years. Of these, 1,260 received concurrent trastuzumab and 589 sequential trastuzumab. Most tumors were grade 3, node positive and ER+. During follow-up 358 RFS events occurred, 231 in the concurrently treated patients compared to 127 in sequentially treated patients. Regarding OS, 290 deaths were observed, 188 deaths in concurrently treated patients compared to 102 deaths in sequentially treated patients, respectively. OS and RFS were similar among sequentially versus concurrently treated patients (adjusted HR 1.11; 95% CI 0.87-1.42; P=0.420 and adjusted HR 1.15; 95% CI 0.92-1.44; P=0.209, respectively).

Conclusion

We observed no significant difference in OS and RFS between patients who received sequential trastuzumab compared to patients treated concurrently. Based on our results no recommendation can be made favoring either of the two treatment sequences for the adjuvant treatment of HER2+ breast cancer patients.
**Title:** Neratinib in the extended adjuvant treatment of patients from Asia with early-stage HER2+ breast cancer after trastuzumab-based therapy: Exploratory analyses from the phase III ExteNET trial

Hiroji Iwata¹, Norikazu Masuda², Sung-Bae Kim³, Kenichi Inoue⁴, Yoshiaki Rai⁵, Takashi Fujita⁶, Zhen-Zhou Shen⁷, Joanne W Chiu⁸, Shoichiro Ohtani⁹, Masato Takahashi¹⁰, Nachito Yamamoto¹¹, Toshiko Miyaki¹¹, Qiang Sun¹², Lu Yen-Shen¹³, Binghe Xu¹⁴, Yoon Sim Yap¹⁵, Aníta Z Bustam¹⁶, Ju Ruey-Jiuan Lee¹⁷, Bo Zhang¹⁷, Richard Bryce¹⁷ and Arlene Chan¹⁸. ¹Aichi Cancer Center, Nagoya, Japan; ²Osaka National Hospital, Osaka, Japan; ³Asan Medical Centre; ⁴Saitama Cancer Center; ⁵Hakuaikai Sagara Hospital, Japan; ⁶Jichi Medical University Hospital, Japan; ⁷Shanghai Cancer Center; ⁸Queen Mary Hospital, Hong Kong; ⁹Hiroshima City Hiroshima Citizens Hospital, Japan; ¹⁰Hokkaido Cancer Center, Japan; ¹¹Chiba Cancer Center, Japan; ¹²Peking Union Medical College Hospital, China; ¹³National Taiwan University Hospital, Taiwan; ¹⁴Chinese Academy of Medical Sciences and Peking Union Medical College, China; ¹⁵National Cancer Centre Singapore, Singapore; ¹⁶University Malaya Medical Centre, Malaysia; ¹⁷Puma Biotechnology Inc and ¹⁸Breast Cancer Research Centre-Western Australia and Curtin University.

**Body:**

**Background:** Current breast cancer knowledge is based largely on studies conducted in western populations. Their findings may not be generalizable to Asian women because of ethnic, genetic and lifestyle differences. Neratinib (N) is an irreversible tyrosine kinase inhibitor of HER1, 2 and 4. The international, randomized, placebo (P)-controlled phase III ExteNET trial showed that 1 year (yr) of N after trastuzumab (T)-based adjuvant therapy significantly improved 2-yr invasive disease-free survival (iDFS) in patients (pts) with early-stage HER2+ breast cancer (HR 0.67; 95% CI 0.50–0.91; p=0.009) [Chan et al. Lancet Oncol 2016]. The significant iDFS benefit with N was shown to be durable after 5 yrs’ follow-up (HR 0.73; 95% CI 0.57-0.92; p=0.008) [Martin et al. ESMO 2017]. We report efficacy and safety findings from pts enrolled from Asian centers (China, Hong Kong, Japan, Korea, Malaysia, Singapore, and Taiwan) on the ExteNET trial to better characterize the effects of N in Asian women.

**Methods:** Pts with early-stage HER2+ breast cancer were randomly assigned to oral N 240 mg/day or P for 1 yr after standard primary therapy and T-based adjuvant therapy. Antidiarrheal prophylaxis was not required by protocol. Data concerning disease recurrences were collected prospectively during yr 1-2 post-randomization, and from medical records during yr 3–5 post-randomization. Primary endpoint: iDFS. HR (95% CI) estimated using Cox proportional-hazards models stratified by nodal status, hormone-receptor status and prior T regimen. Data cut-off: 2-yr analysis, July 2014; 5-yr analysis, March 2017. Clinicaltrials.gov:NCT00878709.

**Results:** Of 2840 randomized pts (N, n=1420; P, n=1420), 341 (12%) were enrolled from Asian centers (N, n=165; P, n=176). Baseline characteristics: median age 53 yr; hormone receptor-positive 48%. Median treatment duration was similar in both groups (N, 351 days; P, 352 days). iDFS events in Asian vs ITT populations are shown in the Table.

<table>
<thead>
<tr>
<th></th>
<th>Primary 2-yr analysis</th>
<th>5-yr analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>P</td>
</tr>
<tr>
<td>Asian population, n</td>
<td>165</td>
<td>176</td>
</tr>
<tr>
<td>iDFS events, n</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>HR (95% CI)b</td>
<td>0.71 (0.31-1.57)</td>
<td>0.54 (0.26-1.08)</td>
</tr>
<tr>
<td>P-value (2-sided)</td>
<td>0.404</td>
<td>0.085</td>
</tr>
<tr>
<td>ITT population, n</td>
<td>1420</td>
<td>1420</td>
</tr>
<tr>
<td>iDFS events, n</td>
<td>67</td>
<td>106</td>
</tr>
<tr>
<td>HR (95% CI)b</td>
<td>0.66 (0.49-0.90)</td>
<td>0.73 (0.57-0.92)</td>
</tr>
<tr>
<td>P-value (2-sided)</td>
<td>0.008</td>
<td>0.008</td>
</tr>
</tbody>
</table>

a. Primary study endpoint; b. Neratinib vs placebo
The incidence of grade 3/4 diarrhea with N was slightly higher in Asian pts (46.1% vs ITT, 39.8%). All other grade 3/4 adverse events with N were rare among Asian pts (elevated ALT, mucosal inflammation, 2 pts each; other events, 1 pt each). Compliance with N in Asian pts was also improved (71% vs ITT, 61%).

**Conclusions:** In Asian pts enrolled into ExteNET, compliance with N was better and the magnitude of N effect was similar or greater that that observed in the ITT population. Although N-related grade 3/4 diarrhea was more common in Asian pts than in the ITT population, all other grade 3/4 events were rare. Despite small pt numbers, our analyses suggest that the findings from ExteNET are applicable to Asian pts, and support the conclusion that N reduces disease recurrences in Asian pts with early-stage HER2+ breast cancer after T-based adjuvant therapy.
Title: Quality of life and patient-reported outcomes in US patients enrolled in the MONALEESA-2 study

Sara M Tolaney, Elizabeth Tan-Chiu, Cristina Truica, Gena Volas-Redd, Mikhail Shtivelband, Anand A Dalal, David Chandiwana and Gabriel Hortobagyi. Dana-Farber Cancer Institute, Boston, MA; Florida Cancer Research Institute, Plantation, FL; Penn State Cancer Institute, Hershey, PA; Northside Hospital, Inc., Atlanta, GA; Ironwood Cancer and Research Center, Chandler, AZ; Novartis Pharmaceuticals Corporation, East Hanover, NJ and University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Background: Improvement to and maintenance of the highest possible health-related quality of life (QoL), in addition to disease control, are key goals of treatment in patients with advanced breast cancer (ABC). Endocrine therapy is preferred as first-line therapy in ABC because of its preferable safety profile compared with chemotherapy. In the MONALEESA-2 study, the cyclin-dependent kinases 4 and 6 inhibitor ribociclib, in combination with letrozole, significantly extended progression-free survival (PFS) compared with placebo + letrozole in patients with hormone receptor–positive (HR+) human epidermal growth factor receptor 2–negative (HER2−) ABC. Patient-reported outcomes demonstrated similar QoL among patients in both treatment groups. Here, we present data from US patients enrolled in the MONALEESA-2 study on overall QoL as well as individual domains.

Methods: Postmenopausal women (N=668) with HR+, HER2− ABC who did not receive prior systemic treatment for ABC and had an Eastern Cooperative Oncology Group performance status score of ≤1, adequate bone marrow and organ function, and no history of active cardiac dysfunction were randomized 1:1 to receive either ribociclib (600 mg/d, 3 weeks on/1 week off) + letrozole (2.5 mg/d, continuous) or placebo + letrozole. The primary end point was locally assessed PFS. Quality of life was reported using the European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire-Core 30 (EORTC QLQ-C30), the EuroQol 5-domain 5 level (EQ-5D-5L) visual analog scale (VAS) of overall health, and the breast symptom score of the EORTC QLQ-Breast Cancer 23 (EORTC QLQ-BR23) module. Data cutoff in this analysis was January 29, 2016.

Results: Patient characteristics and QoL survey reports were well balanced across treatment groups in US patients (n=213). The global health status/QoL scores of the EORTC QLQ-C30 were maintained between groups, and improved over time in the ribociclib group (mean ± standard deviation [SD] score at baseline, 69.1 ± 19.0; at 8 months, 71.3 ± 18.2; and at 16 months, 73.0 ± 16.0) and the placebo group (mean ± SD score at baseline, 69.9 ± 20.0; at 8 months, 75.9 ± 19.2; and at 16 months, 77.0 ± 15.0), which was consistent with scores in the overall population. At 16 months, the proportion of patients who did not experience ≥10% deterioration of all QoL scores was similar among treatment groups.

Quality of Life Outcomes of US Patients in the MONALEESA-2 Study at 16 Months

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Treatment, n</th>
<th>Patients without ≥10% deterioration in score, % (95% CI, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EORTC QLQ-C30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Global health status/QoL</td>
<td>RIB + LET, 26</td>
<td>64.0 (49.4–75.4)</td>
</tr>
<tr>
<td></td>
<td>PBO + LET, 35</td>
<td>50.5 (36.2–63.2)</td>
</tr>
<tr>
<td>Physical functioning</td>
<td>RIB + LET, 21</td>
<td>75.7 (62.6–84.7)</td>
</tr>
<tr>
<td></td>
<td>PBO + LET, 17</td>
<td>73.1 (57.2–83.9)</td>
</tr>
<tr>
<td>Emotional functioning</td>
<td>RIB + LET, 22</td>
<td>71.6 (58.6–81.2)</td>
</tr>
<tr>
<td></td>
<td>PBO + LET, 31</td>
<td>59.7 (45.7–71.2)</td>
</tr>
<tr>
<td>Social functioning</td>
<td>RIB + LET, 17</td>
<td>80.1 (68.5–87.8)</td>
</tr>
<tr>
<td></td>
<td>PBO + LET, 26</td>
<td>60.2 (44.1–73.0)</td>
</tr>
<tr>
<td>EORTC QLQ-BR23 breast symptom score</td>
<td>RIB + LET, 8</td>
<td>88.0 (76.6–94.0)</td>
</tr>
<tr>
<td></td>
<td>PBO + LET, 8</td>
<td>86.2 (70.6–93.9)</td>
</tr>
<tr>
<td>EQ-5D-5L VAS of overall health</td>
<td>RIB + LET, 13</td>
<td>86.6 (76.9–92.4)</td>
</tr>
</tbody>
</table>
LET, letrozole; PBO, placebo; RIB, ribociclib.

Conclusions: Addition of ribociclib to letrozole in US patients enrolled in MONALEESA-2 led to significant prolongation of PFS while maintaining QoL.
The treatment patterns and clinical outcomes of trastuzumab in early human epidermal growth factor receptor 2 (HER2) positive breast cancer in the real-world setting in China

Jihong Guo1,2, Binghe Xu1, Qing Li1, Pin Zhang1, Peng Yuan1, Jiayu Wang1, Fei Ma1, Ying Fan1, Ruigang Cai1, Yang Luo1 and Qiao Li1. 1Cancer Hospital, Chinese Academy of Medical Sciences, Panjiayuan, Chaoyang District, Beijing, China and 2Beijing Chaoyang Hospital, Capital Medical University, Chaoyang District, Beijing, China.

Body: Background: Several prospective interventional studies have proved the efficacy and safety of trastuzumab in adjuvant treatment in HER2 positive breast cancer, while limited data existed to address the treatment patterns and real-world performance of trastuzumab in early HER2 positive breast cancer in China.

Methods: We retrospectively analyzed all the pts who were diagnosed with HER2-positive breast cancer, underwent surgery and received adjuvant treatment in National Cancer Center from 2000 to 2012. The treatment patterns and disease free survival (DFS), overall survival (OS) were analyzed respectively.

Results: A total of 1398 HER2 positive breast cancer pts were identified. The median follow-up time was 79.1 months. 68.5% pts received chemotherapy alone, 3.4% pts only received mono trastuzumab, 28.2% pts received trastuzumab plus chemotherapy. Among 433 trastuzumab treated pts, 64.7% received concurrent trastuzumab with chemotherapy, and 35.3% pts received sequential trastuzumab with chemotherapy.

Baseline characteristics by Chemotherapy alone and Chemotherapy + Trastuzumab.

<table>
<thead>
<tr>
<th>No(%) of patients</th>
<th>Trastuzumab- (N=957)</th>
<th>Trastuzumab+ (N=441)</th>
<th>Total (N=1398)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;=35</td>
<td>96 (10.0)</td>
<td>44 (10.0)</td>
<td>140 (10.0)</td>
</tr>
<tr>
<td>36 - 50</td>
<td>434 (45.4)</td>
<td>244 (55.3)</td>
<td>678 (48.5)</td>
</tr>
<tr>
<td>&gt;50</td>
<td>427 (44.6)</td>
<td>153 (34.7)</td>
<td>580 (41.5)</td>
</tr>
<tr>
<td>Histologic grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>15 (1.6)</td>
<td>7 (1.6)</td>
<td>22 (1.6)</td>
</tr>
<tr>
<td>I-II,II</td>
<td>480 (50.2)</td>
<td>224 (50.8)</td>
<td>704 (50.4)</td>
</tr>
<tr>
<td>II-III,III</td>
<td>285 (29.8)</td>
<td>166 (37.6)</td>
<td>451 (32.3)</td>
</tr>
<tr>
<td>IDC</td>
<td>148 (15.5)</td>
<td>29 (6.6)</td>
<td>177 (12.7)</td>
</tr>
<tr>
<td>Other</td>
<td>29 (3.0)</td>
<td>15 (3.4)</td>
<td>44 (3.1)</td>
</tr>
<tr>
<td>Clinical stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>26 (2.7)</td>
<td>16 (3.6)</td>
<td>42 (3.0)</td>
</tr>
<tr>
<td>I</td>
<td>259 (27.1)</td>
<td>108 (24.5)</td>
<td>367 (26.3)</td>
</tr>
<tr>
<td>II</td>
<td>434 (45.4)</td>
<td>196 (44.4)</td>
<td>630 (45.1)</td>
</tr>
<tr>
<td>III</td>
<td>238 (24.9)</td>
<td>121 (27.4)</td>
<td>359 (25.7)</td>
</tr>
<tr>
<td>Hormone receptor status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>635 (66.4)</td>
<td>256 (58.0)</td>
<td>891 (63.7)</td>
</tr>
<tr>
<td>Negative</td>
<td>322 (33.6)</td>
<td>185 (42.0)</td>
<td>507 (36.3)</td>
</tr>
</tbody>
</table>

Trastuzumab plus chemo had significantly longer DFS compared with chemo alone (85.03% vs 72.15%, hazard ratios, HR=0.531, 95% confidence interval, 95% CI: 0.406-0.696, p<0.001). In addition, the concurrent trastuzumab showed the trend...
with longer DFS compared to the sequential regimen (86.07% vs 82.35%, HR=0.843, 95% CI: 0.515-1.378, p=0.495). Age, tumor size, lymph node status, clinical stage were associated with DFS.

Results of Cox regression hazard model with treatment groups and patient characteristics on DFS

<table>
<thead>
<tr>
<th>Group</th>
<th>HR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concurrent Trastuzumab</td>
<td>0.843</td>
<td>0.515-1.378</td>
<td>0.495</td>
</tr>
<tr>
<td>Sequential Trastuzumab</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemotherapy+Trastuzumab</td>
<td>0.531</td>
<td>0.406-0.696</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Chemotherapy alone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age group (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;=35</td>
<td>1.650</td>
<td>1.198-2.273</td>
<td>0.002</td>
</tr>
<tr>
<td>36 - 50</td>
<td>0.888</td>
<td>0.702-1.122</td>
<td>0.319</td>
</tr>
<tr>
<td>&gt;50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histologic grade</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>I</td>
<td>0.520</td>
<td>0.210-1.288</td>
<td>0.158</td>
</tr>
<tr>
<td>I-II,II</td>
<td>0.504</td>
<td>0.379-0.671</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>II-III,III</td>
<td>0.532</td>
<td>0.390-0.725</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Other</td>
<td>0.514</td>
<td>0.264-0.997</td>
<td>0.049</td>
</tr>
<tr>
<td>IDC</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Clinical stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.261</td>
<td>0.107-0.637</td>
<td>0.003</td>
</tr>
<tr>
<td>I</td>
<td>0.286</td>
<td>0.207-0.395</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>II</td>
<td>0.480</td>
<td>0.379-0.607</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>III</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hormone receptor status</td>
<td></td>
<td></td>
<td>0.022</td>
</tr>
<tr>
<td>Positive</td>
<td>0.773</td>
<td>0.621-0.962</td>
<td>0.021</td>
</tr>
<tr>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

OS was also prolonged in trastuzumab plus chemotherapy compared with chemotherapy alone (93.20% vs 85.45%, HR=0.561, 95% CI: 0.377-0.835, p=0.004).

Conclusions: Our study revealed that, there were still more than half of HER2-positive breast cancer pts couldn't reach anti-HER2 therapy in China, the early stage HER2-positive breast cancer pts could significantly benefit from trastuzumab adjuvant therapy in real-world setting.
Introduction: Triple positive breast cancer is one of intrinsic subtype in breast cancer that is defined namely, ER/PR/Her2 positive tumors. It has multiple strategies for systemic therapy according to the characteristics of hormonal and anti-HER2 responsiveness. Each targeted therapy plays cross-talks between HER-2 and estrogen receptor signaling pathway resulting in endocrine resistance and anti Her2 resistance.


Results: Median follow up duration of 76 months in KBCS cohort (2006-2010) showed the overall survival graph of triple positive breast cancer located in the middle in between Luminal A intrinsic subtype and HER2 enriched subtype \( P<.001 \). Also HER2 directed trastuzumab therapy did not improve the overall survival in triple positive breast cancer patients \( P= .899 \) in contrast to the improved overall survival using trastuzumab therapy in HER2 enriched subtype \( P=.018 \). Like the preceding results, CMC breast cancer data showed the similar results in recurrence free survival \( P<.001 \) and no recurrence free survival improvement using trastuzumab therapy in triple positive breast cancer patient during the median follow up of 33 months \( P=0.800 \).

Conclusion:
1) Anti-HER2 therapy seems less beneficial in Triple positive breast cancer subtype regardless of breast cancer stage.
2) Triple positive breast cancer may require different therapeutic approaches
3) Other targeted agents (mTOR inhibitor, CDK4/6 inhibitor, PIK3CA inhibitor) can be a substitute option for the Trastuzumab therapy in triple positive breast cancer.
**Title:** Final analysis of overall survival (OS) for the epoetin alfa (EPO) phase 3 study, EPO-ANE-3010, of EPO plus standard supportive care (SOC) versus SOC in anemic patients with metastatic breast cancer (MBC) receiving standard chemotherapy

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**Body:** Background: In the interim analysis of study EPO-ANE-3010, for the primary endpoint of progression-free survival (PFS), the non-inferiority objective in ruling out a 15% increased risk in progressive death (PD) or death per investigator-determined PD was not achieved (JCO 34:1197-1207, 2016). PFS, based on independent review committee (IRC)-determined PD, however, met the non-inferiority criteria. At the interim analysis, OS with 1,337 deaths was reported; we now report the final analysis at 1,653 deaths and the updated PFS.

Methods: This multinational (19 countries and 132 participating sites), phase 3, randomized, open-label noninferiority study included anemic (≤11.0 g/dL hemoglobin) women receiving first- or second-line standard chemotherapy for MBC (Eastern Cooperative Oncology Group performance status of 0 or 1). Subjects were randomized (1:1) to receive either standard SOC for treatment of anemia plus EPO (40,000 IU subcutaneous) weekly up to 4 weeks after the last dose of cytotoxic chemotherapy, or SOC alone. The primary endpoint was PFS (using Cox's regression model). Secondary endpoints included OS, time to tumor progression (TPP), overall response rate (ORR) and safety assessments.

Results: A total of 2,098 subjects were enrolled (EPO plus SOC: n=1,050; SOC alone: n=1048). Demographic and baseline characteristics were well-balanced across the groups; median age was 52 years, most were white (67.5%) or Asian (30.5%) and median BMI was 26.0 kg/m². Primary efficacy analysis (based on investigator-determined PD) showed a median PFS of 7.4 months for both groups (hazard ratio [HR], 1.094; 95% CI: 0.996, 1.201); upper bound exceeded prespecified noninferiority margin of 1.15. A 9% increased risk for PD/death in the EPO plus SOC group was observed and did not statistically rule out a 15% increased risk. Median PFS per IRC-determined PD was 7.6 months in both groups (HR, 1.028; 95% CI: 0.922, 1.146), this met pre-defined non-inferiority margin of 1.15 with a 3% risk increase in PD/death in EPO plus SOC group. At the final analysis for OS, median OS was 17.8 months in the EPO plus SOC group and 18.0 months in the SOC group; HR: 1.073 (95% CI: 0.974, 1.182); median TPP was 7.5 months in both groups (HR, 1.099; 95% CI, 0.998 to 1.210), and ORR was 50% in the EPO plus SOC group and 51% in the SOC group (odds ratio, 0.939; 95% CI, 0.789, 1.117). Red blood cell (RBC) transfusions were 5.8% versus 11.5% (P<0.001), and thrombotic vascular events were 2.8% versus 1.4% (P=0.038), respectively, in EPO plus SOC group and SOC group. The incidence of death due to PD were similar in both groups (EPO plus SOC: 93%; SOC: 91%).

Conclusion: The primary endpoint, PFS based on investigator-determined PD, did not meet noninferiority criteria but for PFS based on IRC-determined PD, noninferiority criteria was met. Overall, this study did not statistically rule-out a 15% increased risk in PD/death. The final analysis did not show statistically different OS in the EPO plus SOC group versus the SOC group. No new safety signals were noted with EPO treatment and the results are consistent with the known safety profile of EPO.
2017 San Antonio Breast Cancer Symposium

Publication Number: P1-14-02

Title: Impact of loco-regional treatment (LRT) on overall-survival (OS) in patients with de novo metastatic breast cancer (MBC): Results of the French ESME multicenter national observational programme


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Body: Background

Previous retrospective studies and meta-analysis suggest an improved overall survival (OS) brought by loco-regional treatment (LRT) of primary tumor in de novo metastatic breast cancer (MBC) patients (pts), while prospective trials have mixed results. Most of the previous studies recruited pts before 2005 and consequently did not include recent medical therapies and advances used in MBC.

Patients and methods

The ESME database include individual data from MBC pts diagnosed between 2008 and 2014, who initiated their treatment in 1 of the 18 participating French comprehensive cancer centers. Our first aim was to study the impact of LRT, defined as surgery, radiotherapy or both, on OS in de novo MBC pts, defined as pts diagnosed with synchronous metastasis or within 90 days from diagnosis of primary tumor. OS was calculated from the date of diagnosis of metastatic stage and was estimated by the Kaplan Meier method. Univariate and multivariate analyses were performed using Logrank-test and Cox-model, respectively.

Results

Of the total ESME cohort (16703 pts), 4507 (26.9%) were diagnosed with de novo MBC and 4276 fulfilled inclusion criteria for the present study. Median age was 60 years. 66.3% pts had T3-T4. 13.1% (n=495) had triple-negative (TN) BC, 24.4% (n=918) HER2+ BC and 62.5% (n=2536) HR+/HER2- BC. Sites of metastases were bone (69%), liver (30.9%), nodes (29.5%) and lung (25.7%). 77.4% had less than 3 sites involved by metastatic disease.

Of 4276 pts, 1706 (39.9%) received a LRT. Among them, 444 (26%) and 535 (31.4%) had surgery or radiotherapy alone respectively, 727 (42.6%) had a combination of both. 99% of pts received a systemic treatment: hormone therapy for 60.3% and chemotherapy +/- targeted therapy for 72.8% of them. Compared with pts with no LRT, pts in the LRT group were younger (median age: 57 vs 61 years, p<0.0001), had smaller tumor (40.4% ≤ T2 vs 28.2%, p<0.0001) with more N0 status (26.5% vs 19.3%, p<0.0001), and different phenotypes: TN 12.1% vs 13.8%; HER2+ 26.4% vs 23.1%; HR+/HER2- 61.5% vs 63.1% (p=0.0447). Metastatic disease in the LRT group was more likely to be limited to 1 or 2 sites (86.4% vs 71.4%), with more non-visceral sites (52.3% vs 36.5%).

With a median follow-up of 45.3 m, median OS and initial PFS for the whole population were 45.2 m [95%CI: 43.3-47.1] and 13.8 m [95%CI: 13.2-14.4], respectively. Median OS for TN, HR+/HER2- and HER2+ pts were 19 m [95%CI:17-21], 47.4 m [95%CI: 45.2-50.4] and 53.3 m [95%CI: 48.9-60.2], respectively. By multivariate analysis, LRT was an independent prognostic factor for OS (HR=0.76; 95%CI: 0.64-0.89; p=0.001), together with age, histological subtype, number and patterns of metastatic sites. More advanced analyses will be presented in December. </del>

Conclusion

As in older series, this work finds that de novo MBC pts treated after 2008 may derive a prolonged OS, extending up to 4 years for HR+ and HER2+ subgroups. Given the classical prescription biases in such retrospective works, this should be carefully interpreted but might help in better selecting those pts for whom such strategy would be beneficial.
**Title:** ABRAZO: Exposure-efficacy and -safety analyses of breast cancer patients with germline *BRCA1/2* mutations receiving talazoparib in a phase 2 open-label trial

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**Body:**

**Background:** Talazoparib (TALA) is a dual-mechanism poly (ADP-ribose) polymerase (PARP) inhibitor that traps PARP on DNA. Efficacy results of this phase 2 trial were previously presented (Turner et al, ASCO 2017, abstract 1007). This study included sparse pharmacokinetic (PK) sampling for patients through cycle 4 of therapy. Exploratory analyses included assessment of exposure versus parameters of efficacy and safety.

**Methods:** ABRAZO (NCT02034916) was a parallel-cohort, open-label phase 2 study of TALA (1 mg/d) following (i) platinum-based therapy (cohort 1) or (ii) ≥3 platinum-free cytotoxic-based regimens (cohort 2) in patients with locally advanced or metastatic breast cancer and germline *BRCA1/2* mutation. Sparse PK sampling was performed on day 1 of cycles 1-4, consisting of a predose sample collected ≤60 minutes prior to dosing and 2 postdose samples collected ≥30 minutes after dosing (time of food ingestion prior to the dose was collected). The collection times of the 2 postdose samples were separated by ≥2 hours. Efficacy parameters included radiographic progression-free survival (rPFS) by central review and objective response rate (ORR). Safety parameters included incidence of overall adverse events (AEs) and grade ≥3 AEs. Individual AUCs (area under concentration-time curves) for exposure-response analyses were predicted by population PK analyses.

**Results:** Patients were divided into AUC tertiles: low (median, 109.0 ng·hr/mL; n=27), intermediate (median, 170.8 ng·hr/mL; n=27), and high (median, 219.2 ng·hr/mL; n=27). Median rPFS was 5.3 months (95% confidence interval [CI], 3.1, 8.3) in the lowest AUC tertile, 5.6 months (95% CI, 3.7, 8.4) in the intermediate AUC tertile, and 5.3 months (95% CI, 3.9, 5.6) in the highest AUC tertile. The ORR was 22.2% (95% CI, 8.6, 42.3) in the lowest AUC tertile, 25.9% (95% CI, 11.1, 46.3) in the intermediate AUC tertile, and 37.0% (95% CI, 19.4, 57.6) in the highest AUC tertile. AEs of any grade were reported in 11 patients (40.7%) in the lowest AUC tertile, 21 patients (77.8%) in the intermediate AUC tertile, and 22 patients (81.5%) in the highest AUC tertile. Grade ≥3 AEs were reported in 8 patients (29.6%) in the lowest AUC tertile and in 18 patients (66.7%) in the intermediate and highest AUC tertiles. The most common AEs in all 3 exposure tertiles were anemia, thrombocytopenia, and neutropenia.

**Conclusions:** Median rPFS did not change with increasing systemic exposure. There may be a trend to higher ORR in patients with highest systemic exposure. A larger percentage of patients experienced AEs with elevated systemic exposure. Increased response rates with greater exposure does not translate to improved rPFS. These results should be interpreted with caution due to the low patient numbers in each cohort.
**Body:** BACKGROUND - Metastatic breast cancer (MBC) is a life-threatening disease. It is important to provide data about real-life MBC patients (pts) to understand the current prognostic factors. The aim of the present observational study, named COSMO (Checking Overall Survival in a MBC Observational study) is to describe the overall survival (OS) in a large cohort of MBC pts, assessing its correlation with specific prognostic factors (demographic, clinic, pathologic and biological).

**PATIENTS AND METHODS** - The COSMO study is a multicenter, retrospective, cohort study, developed throughout the collaboration of 31 Italian oncological centers. Data about pts diagnosed as metastatic from 01/01/2000 to 31/12/2008, were collected. The association between molecular subtypes, metastatic sites, disease free interval (DFI) and OS were assessed. Pts were classified in three subgroups, based on the biological characteristics of their tumor: luminal, HER2-positive (regardless of hormone receptor) and triple negative (TN). Metastatic sites were categorized as visceral versus non-visceral disease, only bone and central nervous system (CNS) metastases. DFI was calculated from diagnosis to first relapse only for M0 pts.

**RESULTS** - Of 3931 MBC pts enrolled in the study, 3720 were evaluable, with a median age of 61 years (interquartile range, IQR, 51-71). 1804 (62,1%) pts had a luminal disease, 691 (23,8%) HER2-positive, 410 (14.1%) TN. Median DFI was 3.2 years (IQR 1.7- 6.0). Regarding metastatic sites, pts with visceral disease were 2332 (63%); 826 (22,2%) pts had bone isolated metastases; in 306 (8,3%) pts, CNS metastases were reported. With a median follow up of 9 years (IQR 5.7-11.0) and 3098 (83.3%) recorded events, we founded a median OS of 2.8 years (95%CI: 2.7-2.9) years. OS was strictly depending from molecular subtypes with a better prognosis for HER2-positive versus luminal and TN MBC pts, median OS of 3.1 (95%CI 2.8-3.4), 3.0 (95%CI: 2.9-3.1) and 1.5 (95%CI: 1.3-1.7) years respectively (p-value<0.001). 525 (14,1%) pts received trastuzumab. Metastatic sites affect prognosis, with a better OS for bone disease (3.4 years, 95%CI: 3.1-3.6) versus visceral disease (2.2 years 95%CI: 2.0- 2.3). Brain metastasis correlate with the worst prognosis: OS of 1.5 years (95% CI: 0.8 – 1.7). Even DFI shows a correlation with prognosis: pts with DFI>2 years show a median OS of 3 years (95% CI: 2.9 – 3.2), while those with DFI<2 years have a median OS of 2.4 years (95% Cl: 2.3-2.6); HR was 0.69 (95%CI: 0,62-0,76) for every five years of increase in DFI (p-value<0.001).

**CONCLUSIONS** - Molecular subtype is crucial for prognosis: HER2-positive subtype has the best prognosis, while TN subtype has the shorter OS. Having a longer DFI from diagnosis (>2 years) correlate with a better prognosis. Our study confirm that sites of metastasis affects outcome: visceral involvement correlates with poor prognosis and, particularly, pts with brain metastasis represent the worst subgroup, while pts with solely bone disease have the best prognosis. The COSMO study provides a view on the Italian landscape of MBC between 2000 and 2008, adding new insights about pts prognosis.
### Title: Ribociclib dose recommendations for potential pharmacokinetic drug interaction and in special patient populations with organ impairment

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### Body: Background:
Ribociclib (Kisqali®) is a CDK4/6 inhibitor, recently approved in the U.S. as a first line therapy for HR+/HER2- advanced breast cancer (ABC) in combination with an aromatase inhibitor. The recommended starting dose is 600 mg/day orally (3 wks-on/1 wk-off). Ribociclib inhibits CYP3A4 and its metabolism is mostly CYP3A4-mediated. Here we evaluate the effect of food, conmeds and special patient populations on ribociclib pharmacokinetics (PK) and dose recommendations.

### Methods: In vitro and clinical data (food effect, drug–drug interactions, organ impairment and Phase I/III trials) were analyzed using physiologically-based PK (PBPK), population PK (PopPK) models and non-compartmental analysis (NCA). Dose recommendations were made considering ribociclib PK, safety and efficacy data.

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<th>Food effect/Conmeds/Special populations</th>
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<tr>
<td>Food effect</td>
<td>Evaluate food effect on ribociclib PK following single oral dose (SD) 600 mg in healthy volunteers (HV)</td>
<td>No changes in ribociclib PK when taken with food</td>
<td>Ribociclib can be taken with or without food</td>
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<tr>
<td>Gastric pH-elevating Agents</td>
<td>-</td>
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<td>CYP3A4 Inhibitors</td>
<td>Evaluate effect of ritonavir on SD ribociclib PK in HV</td>
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</tr>
<tr>
<td>CYP3A4 Inducers</td>
<td>Evaluate effect of rifampicin on SD ribociclib PK in HV</td>
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<td>Strong inducers: avoid -Moderate inducers: no dose modification</td>
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<tr>
<td>CYP1A2 Substrates</td>
<td>Evaluate effect of multiple dose ribociclib 400 mg on caffeine PK</td>
<td>No effect of ribociclib on caffeine</td>
<td>No dose adjustment</td>
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|                     | in HV                                                                 | Similar exposure in mild and ~30% higher in moderate and severe HI compared to normal hepatic function | Mild HI: No dose adjustment  
Moderate & severe HI: reduce starting dose to 400 mg |
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<td>Evaluate ribociclib 400 mg SD PK in non-cancer subjects with varying degrees of HI</td>
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<tr>
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<td>No effect of mild and moderate RI on ribociclib exposure (PopPK), study in severe RI ongoing</td>
<td>-Mild or moderate RI: No dose adjustment - Severe RI: results pending</td>
</tr>
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**Conclusions:** Ribociclib 600 mg daily (3 wks-on/1 wk-off) has shown clinical benefit in treatment of HR+/HER2- ABC with a manageable safety profile. Ribociclib can be conveniently administered without regard to food intake or gastric pH-elevating agents. Appropriate ribociclib dose recommendations with concomitant medications or organ function impairment were made considering ribociclib PK, safety and efficacy.
Title: Selective internal radiation therapy (SIRT) with Yttrium-90 resin microspheres and FOLFOX/5FU chemotherapy in pre-treated breast cancer patients with liver metastases: A retrospective analysis of response rates, times to progression and survival of patients treated in Manchester UK between 2010 and 2016

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Body: Background:
SIRT is a globally licensed technique of Radio-Embolization (RE) of hepatic tumors via intra-arterial infusion of β-particle emitting Yttrium-90 (Y-90) radio-labelled microspheres. It increases response rates and hepatic time to progression in metastatic colorectal cancer when used in combination with 5FU/Oxaliplatin (FOLFOX) chemotherapy with acceptable toxicity profile. FOLFOX gives a radio-sensitizing effect and also controls disease outside the liver. Breast cancer liver metastases (BCLM) patients often have extra-hepatic disease and respond to multiple lines of systemic therapy and SIRT is infrequently used.

Methods and patients
Between 2010 and 2016 we treated 25 BCLM patients with Y-90 SIRT. Receptor status: 20 ER+ve/HER-2 -ve, 3 ER-ve/HER-2 +ve, 2 triple -ve. Eleven patients had liver only disease with 14 also having known extra-hepatic disease. Average number of previous lines of therapy in metastatic setting: chemotherapy = 2.4; endocrine = 1. Sixty-four % patients had prior Capecitabine (n=16); 12% platinum (n=3, all Carboplatin). Twenty patients received chemotherapy with SIRT: 17 had modified FOLFOX6 (Oxaliplatin/bolus 5FU day1, infusional 5FU day 1-3 (46 hrs); 3 patients had Modified de Gramont style 5FU alone. Five patients had no chemotherapy.

Sir-spheres were inserted on day 2 of FOLFOX with the 5FU infusion pump continuing to day 3. Further 2-weekly FOLFOX chemo cycles were at clinician's discretion: average number delivered 3.8. Four patients had the liver treated in two halves, approximately 6 weeks apart. One patient received SIRT only to half the liver. Patients were imaged with PET-CT/CT before and 2-3 months after SIRT. Retrospective case note review was performed and data correlated to evaluate tumor response (RR); hepatic and extra hepatic progression free survival (HPFS and EHPFS) and overall survival (OS). Accurate toxicity data was not recorded.

Results
Hepatic CT response rates: PR 56% (n=14), SD 28% (n=7) and PD 16% (n=4). Hepatic PET response rates: CR 32% (n=8), PR 40% (n=10), SD 12% (n=3), PD 16%(n=4). (Overall PET liver disease control rate = 84%).

Eight patients (32%) had extra-hepatic PD at first assessment. Of them, 4 had PR, 2 SD and 2 PD in the liver at that assessment. Two HER-2 +ve patients had brain metastases as first sign of PD within 75 days, an area not previously screened. Of 16 pre-treated with Capecitabine, liver CT response rates: 62.5% PR, 18.75% SD (n=10,3). Post SIRT/FOLFOX, average number of therapy lines: 2 for chemo and 0.75 for endocrine, with 8 patients still alive at time of censoring.

Median OS: 766 HPFS: 210 days (CI 140-286). Median EHPFS in patients with extra-hepatic disease: 152 days (CI 96-636).

Conclusions
SIRT with FOLFOX in previously treated BCLM patients produces high response rates, excellent tumor control and time to progression in the liver with good overall survival. It does not seem to decrease the ability to give further lines of chemotherapy and can be considered as an option for breast cancer patients with liver metastases.
2017 San Antonio Breast Cancer Symposium

Publication Number: P1-15-01

Title: Final analysis of SWOG S0230/Prevention of early menopause study (POEMS)

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Body: BACKGROUND: The SWOG S0230/POEMS study demonstrated a 70% reduction in ovarian failure (OF) with goserelin coadministration during chemotherapy (CT) for ER-negative early breast cancer (BC; Moore H et al, NEJM 2015). Goserelin use was also associated with more pregnancies as well as favorable disease free survival (DFS) and overall survival (OS). Here we report the final analysis after 5 years of follow-up.

METHODS: Premenopausal women age <50 with stage I-IIIA ER/PR-negative BC to be treated with cyclophosphamide-containing CT were randomized to receive standard CT with or without monthly goserelin 3.6 mg SQ starting at least 1 week prior to the first CT dose. The primary endpoint was OF at 2-years, defined as amenorrhea for the prior 6 months and post-menopausal FSH. Secondary endpoints included pregnancies, disease free survival (DFS) and overall survival (OS). An unplanned analysis of rate of menses recovery at 2 years (presence of menses within 6 months of the 2 year time-point or pregnancy within the first 2 years) was also conducted. OF and pregnancy endpoints were analyzed using multivariable logistic regression adjusting for stratification factors (age and CT regimen); DFS and OS were examined using multivariable Cox regression, adjusting for stratification factors and stage. Two-sided p-values are reported unless otherwise specified in accordance with protocol design.

RESULTS: Among 257 randomized participants, 218 were eligible and evaluable. One hundred thirty-six eligible and evaluable patients had OF data and 186 had menstrual data. Median age was 37.7 years. Among the 136 patients with OF data, the odds ratio (OR) for OF at 2 years was 0.30 (95% CI 0.1-0.98; one-sided p=0.023) comparing CT with goserelin to standard CT alone. Among 186 patients with menstrual data, 80% recovered menses by 2 years in the goserelin arm compared with 70% in the standard arm (OR=1.74, 95% CI: 0.83-3.66, p=0.15). Pregnancies, DFS and OS are reported for all 218 eligible and evaluable patients. With a median follow-up of 5.1 years, 22% of patients in the goserelin group had at least one pregnancy compared with 12% in the standard group (OR 2.38, 95% CI 1.08-5.26, p=0.03). Cumulative incidence of pregnancy at 5 years is 23% in the goserelin arm compared with 12% in the standard group. Five-year Kaplan-Meier DFS estimates are 88% in the goserelin arm compared with 79% in the standard arm (HR=0.50, p=0.05). Five-year OS is 92% with goserelin versus 83% in the standard arm (HR=0.47, p=0.06). Including all 257 randomized patients, HR for DFS and OS are 0.67 and 0.48 (p=0.18 and p=0.05).

CONCLUSION: Ovarian suppression with goserelin during chemotherapy for hormone receptor-negative breast cancer reduces OF risk and, after 5 years of follow-up, continues to be associated with more pregnancies and improved survival compared with chemotherapy without goserelin.

SUPPORT: NIH/NCI grant awards CA189974, CA180888, CA180819, CA074362; AstraZeneca
**Title:** Benefit of adjuvant chemotherapy in disease-free survival for T1N0 triple negative breast cancer

Zaida Morante\textsuperscript{a}, Gabriel De la Cruz-Ku\textsuperscript{b}, Joseph Pinto\textsuperscript{c}, Jhajaira Araujo\textsuperscript{c}, Hugo Fuentes\textsuperscript{d}, Daniel Enriquez\textsuperscript{d}, Renato Luque\textsuperscript{a}, Eduardo Eyzaguirre\textsuperscript{a}, Antonella Saavedra\textsuperscript{a}, Maria Luján\textsuperscript{a}, Silvia Neciosup\textsuperscript{d} and Henry Gomez\textsuperscript{d}. \textsuperscript{a}Universidad Cientifica del Sur, Lima, Peru; \textsuperscript{b}Instituto Nacional de Enfermedades Neoplasicas, Lima, Peru and \textsuperscript{c}Oncosalud - AUNA, Lima, Peru.

**Body:**

**Background:** Although chemotherapy is not recommended in low-risk early breast cancer, triple-negative breast cancers (TNBC) have twice risk of recurrence despite an aggressive treatment. In this study we evaluated the role of chemotherapy in the outcome of T1N0 TNBC patients in terms of disease-free survival (DFS) and overall survival (OS).

**Methods:** We evaluated a retrospective cohort T1N0 TNBC patients diagnosed between 2000 to 2014 at the Instituto Nacional de Enfermedades Neoplasicas (Lima-Peru). Survival rates differences were assessed by Log-rank test and prognostic factors were identified using the Cox proportional hazards model.

**Results:** We identified 123 T1N0 TNBC patients. The median age was 51 years (range: 28-85), where 38.5% were premenopausal. Thirty-six (29.3%) were pT1a/b and 87 were pT1c (70.7%). ACT was administered more frequently in pT1c patients (74.7% vs 55.6%; P=0.036). Locoregional relapse and distant metastases rates were 8.3% vs 19.5% and 5.6% vs 16.1% for tumors pT1a/b and pT1c, respectively. The median of follow-up was 8.8 years. Independent prognostic factors were pT stage and treatment with ACT. In relation to pT stage, DFS rates were 97% vs 78% (at 5-years) and 97% vs 70% (at 8-years) for pT1a/b vs pT1c patients, respectively (HR=4.8; 95%CI:1.46-17.0; P=0.015). In the other hand, patients treated with ACT had a better outcome with DFS rates to 5-years of 86% vs 74% and 8-years of 81% vs 65% (HR: 0.41, 95%CI: 0.17-0.97, p=0.043). Our study had not statistical power to evaluate benefit of ACT in pT1a/b patients. In cases with pT1c tumors, treatment with ACT was the only factor associated with a better DFS (HR=0.29, 95%CI: 0.11-0.77, p=0.012). Because the low mortality in our cohort, OS was not evaluable.

**Conclusions:** Treatment with adjuvant chemotherapy reduces the recurrence risk in 71% among pT1cN0 TNBC.
Management and outcomes of metaplastic breast cancer

Horatio Thomas¹, Nora Horick², Laura M Spring², Elena F Brachtel² and Rachel B Jimenez². ¹Harvard Medical School, Boston, MA and ²Massachusetts General Hospital, Boston, MA.

Body: Purpose/Objectives: Metaplastic breast cancer (MBC) is a rare malignancy composed of both epithelial and mesenchymal components that accounts for less than 1% of primary breast carcinomas. Knowledge of effective management of the disease remains limited. We retrospectively evaluated the treatment and outcomes of patients with MBC from three academic hospitals.

Materials/Methods: Patients diagnosed with MBC between June 2, 1993 and May 6, 2016 were identified. Demographic and clinical variables were extracted via primary chart review. Descriptive statistics were utilized to summarize the patient cohort's clinical course. The Kaplan-Meier method was used to obtain estimates of local control (LC) and survival.

Results: Seventy-six patients were identified with a median follow-up of 7.6 years (range: 0.18-19.9 years). The median age at diagnosis was 54 (range 28-81). About two-thirds of patients (67%, n=51) presented with a palpable mass while the remaining patients were screen-detected via mammogram (32%, n=24). The majority of patients were AJCC-7 stage I (38%, n=29) or stage II (49%, n=37), while 10% (n=8) were stage III and 3% (n=2) stage IV. About half of tumors (46%, n=35) were subtyped using WHO histologic classification of MBC. Over half of subtyped cases were spindle cell carcinoma (51%, n=18), 17% were matrix-producing carcinoma (n=6), 23% were adenocarcinoma with squamous differentiation (n=8), 6% were carcinosarcoma (n=2), and 3% were mixed (n=1). The majority of patients had triple negative disease (82%, n=62), while 13% (n=10) had HR+/HER2- disease, and 5% (n=4) had HER2+ disease. Most patients had high grade tumors (84%, n=64) and received breast conserving surgery (61%, n=46) while 39% (n=29) had mastectomies. Seventy-six percent (n=58) of patients received chemotherapy and 61% (n=46) received radiation therapy. All HR+ patients received adjuvant endocrine therapy, and 1 patient received immunotherapy. Of patients who received chemotherapy, 78% (n=45) received adjuvant therapy alone, 17% (n=10) neoadjuvant therapy alone, and 5% (n=3) both. Seventy percent (n=41) of chemotherapy regimens included a taxane. Among 74 patients without metastatic disease at presentation, recurrences were observed in 18% (n=13). Most patients recurred distantly (69%, n=9), while the remainder had isolated local recurrences (n=4). Of 9 distant failures, 3 had MBC subtype information and all 3 were spindle cell carcinoma. Of 4 local recurrences, 3 of 4 were adenocarcinoma with squamous cell differentiation and 1 was spindle cell carcinoma. At a median follow-up of 7.6 years, the local recurrence-free survival was 88%, disease-free survival was 80%, and overall survival was 80%. Kaplan-Meier point estimates for remaining free of local recurrence versus distant recurrence were 99% (95% C.I., 90-100) versus 96% (95% C.I., 87-99) at 2 years and 88% (95% C.I., 76-94) versus 87% (95% C.I., 75-93) at 5 years.

Conclusions: MBC is a rare histologic subtype that commonly presents with high-grade disease and triple-negative receptor status. In contrast to other smaller series, local and distant failure rates in this cohort were consistent with non-MBC triple negative cohorts. Additional molecular based research is warranted to further characterize features associated with local and distant failure.
**Title:** Outcome of small (≤1 cm), node-negative breast cancer

Mahvish Muzaffar\(^1\), Praveen Namireddy\(^1\), Rafaeh Naqash\(^1\), Jan Wong\(^1\) and Nasreen Vohra\(^1\). \(^1\)Brody School of Medicine/East Carolina University, Greenville, NC.

**Background:** Screening mammogram has resulted in increased diagnosis of very small breast cancers, especially less than 1 cm node negative. These small tumors have excellent prognosis with cancer-specific survival rates as high as 90% to 95%. This study evaluates outcome in different subtypes of very early breast cancer in a national population database.

**Method:** Patients with stage I breast cancer, tumor ≤1 cm with negative nodes (T1aN0 (<0.5cm), T1bN0 (≥0.5cm to ≤1 cm)) diagnosed between 2006 and 2011 were identified in the SEER database. We excluded patients with missing biomarker information. Treatment outcome and prognostic factors for disease-specific survival (DSS) and overall survival (OS) were evaluated.

**Results:** We identified 70,543 cases and included 54,796 patients with stage T1aN0M0 and T1bN0M0 in the final analysis. The mean age was 62.09 yrs. (CI 95% 62.2-61.99), 84% are white, 7% black and 7% others. 89% had ER positive tumor, 11% ER negative and 3% had Her 2 positive tumors. 71% of patients had T1b (≤1 cm). The 5-year disease specific survival (DSS) and overall survival (OS) for patients with stage T1aN0, T1bN0 was 98.7% and 93.7%, respectively. Estrogen receptor (ER) positive tumors were associated with improved 5-yr DSS 99% vs. 96% in ER negative (p<0.0001) and OS in ER positive 94% vs. 92% (p<0.0001). Among white patients 5-yr DSS was 98.8% and OS was 93.7% while 5yr-DSS was 94%, OS 91.5% among black vs. 5-yr DSS 99% and OS 96.3% in others (Asian or Pacific Islanders, AI), (p<0.0001). Tumor subtype was not associated with significant difference in outcome but T1a tumor was associated with OS 94.5% vs. 93.4% with T1b tumors (p<0.0001) On cox model analysis factors which correlated with prolonged DSS and OS are race (p<0.0001), older age (p<0.0001), ER positivity (p<0.0001) and tumor less than 5mm (p=0.0006).

**Conclusions:** Very early breast cancer is associated with excellent outcome but has some heterogeneity. Nonwhite/Non Black race was associated with better survival compared to white and black patients. ER positive tumors, and older age were also associated with better outcome. This data while reassuring also brings into question the overtreatment of this disease subset. One of the limitations of this dataset is lack of details of systemic therapy administered. Conventional prognostic factors are not sufficient to risk stratify very early breast cancer and molecular profiling may help identify patients who will need adjuvant treatment.
2017 San Antonio Breast Cancer Symposium

Publication Number: P1-15-05

Title: Oncologists’ perception of anti-estrogen therapy benefit

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Body: Background
Recent publication of Suppression of Ovarian Function (SOFT) and Tamoxifen and Exemestane (TEXT) trials provides additional options for premenopausal women with hormone receptor (HR)-positive breast cancer in improving estrogen blockade - tamoxifen (T) plus ovarian function suppression (OFS) and aromatase inhibitors (AI) plus OFS. Analysis of these trials conclude that, for a premenopausal woman with HR-positive breast cancer with high risk features for recurrence have an absolute benefit for five-year breast cancer free interval (BCFI) would be 5% with the use of T + OFS and 10 - 15% with Al + OFS compared to the use of T-alone. However, therapeutic option decision-making is dependent on the oncologists’ perception of the benefit and risk for adjuvant anti-estrogen therapy. We aimed to evaluate an oncologist's perception of the absolute benefit for anti-estrogen therapy options in HR–positive breast cancer and identify predictors for variations in perceptions of benefit.

Methods
We designed a survey using a clinical vignette of a young, premenopausal woman with stage IIIA HR-positive breast cancer with high risk of recurrence. After obtaining Institutional Review Board (IRB) approval from the University of Kentucky, we sent online and paper survey forms to a convenience sample of 510 oncologists in the United States. Using a scale between 0 and 100, the oncologists were asked to estimate the five-year BCFI (first occurrence of invasive locoregional, distant or contralateral breast cancer) for a woman with similar characteristics if treated with the following: 1) lumpectomy (L) + Radiation (RT); 2) L + RT + chemotherapy (C); 3) L + RT + C + T x five years; 4) L + RT + C + T + OFS; and 5) L + RT + C + AI + OFS. Baseline demographics such as gender, practice setting (academic vs community), years in practice and proportion of breast cancer patients in practice were also collected.

Results
The highest estimated mean five-year BCFI for the study patient was 76.5% with the addition of AI + OFS to L + RT + C. The estimated absolute benefit for anti-estrogen therapy options: T + OFS vs T-alone was 3.4% (95% CI: 2.7 – 4.0) and for AI + OFS vs T-alone was 5.9% (95% CI: 5.1 – 6.7).

Oncologists' Perception of Absolute Benefit Therapy in Premenopausal Women with Breast Cancer

<table>
<thead>
<tr>
<th>Anti-Estrogen Therapy</th>
<th>5-year BCFI (mean)</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>L + RT</td>
<td>43.6 +/- 18.6</td>
<td></td>
</tr>
<tr>
<td>L + RT + C</td>
<td>59.5 +/- 15.5</td>
<td></td>
</tr>
<tr>
<td>L + RT + C + T x 5 year</td>
<td>70.6 +/- 13.4</td>
<td></td>
</tr>
<tr>
<td>L + RT + C + T + OFS</td>
<td>74.1 +/- 12.9</td>
<td></td>
</tr>
<tr>
<td>L + RT + C + AI + OFS</td>
<td>76.5 +/- 12.7</td>
<td></td>
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</tbody>
</table>

There was no difference in perception of benefit with these anti-estrogen therapy options by gender, practice setting, years in practice or proportion of breast cancer patients seen in practice.

Discussion
Regardless of their gender, practice setting, clinical experience or volume of breast cancer patients, oncologists significantly underestimate the absolute benefit for various estrogen blockade therapies when compared to the estimated benefit of data from randomized, controlled SOFT and TEXT clinical trial data. We highlight an area for improvement in quality of care that offers an immediate impact on positive outcomes for large numbers of premenopausal women with breast cancer.
Localized triple negative breast cancer in extremes of life

Zaida Morante\textsuperscript{2}, Gabriel De la Cruz-Ku\textsuperscript{1}, Joseph Pinto\textsuperscript{3}, Daniel Enriquez\textsuperscript{2}, Antonella Saavedra\textsuperscript{1}, Maria Luj\text{\~n}\textsuperscript{1}, Renato Luque\textsuperscript{1}, Eduardo Eyzaguirre\textsuperscript{1}, Hugo Fuentes\textsuperscript{2}, Silvia Neciosup\textsuperscript{2} and Henry Gomez\textsuperscript{2}. \textsuperscript{1}Universidad Cientifica del Sur, Lima, Peru; \textsuperscript{2}Instituto Nacional de Enfermedades Neoplasicas, Lima, Peru and \textsuperscript{3}Oncosalud - AUNA, Lima, Peru.

Body: Background: Triple-negative breast cancer (TNBC) in young and older patients has specific features and outcomes. While in younger patients, it is characterized by being aggressive with worse survival, in advanced ages it may have a better outcome which reasons have not yet fully elucidated. Our objective was to compare clinicopathological characteristics, treatment and survival rates between very young with elderly population diagnosed of TNBC.

Methods: We conducted a retrospective study analysis of patients with TNBC at clinical stages I-III between 2000 to 2014 at "Instituto Nacional de Enfermedades Neoplasicas". A total of 273 medical records were reviewed, our patients were divided in very young (≤35 years) and older (≥65 years) population. The Kaplan-Meier method was conducted to compare Disease-free survival (DFS) and Overall Survival (OS) by age and chemotherapy, while Cox regression analysis assessed hazard ratios.

Results: From 273 patients with TNBC, 132 were ≤35 years, and 141 were ≥65 years. Elderly population had more obesity (13.1% vs 23.6%, \textit{p}=0.03) and comorbidities (15.9% vs 46.1%, \textit{p}<0.001); 42 (15.4%) had antecedent of breast and/or ovarian cancer (≤35y vs. ≥65y - 18.2% vs. 12.8% - \textit{p}=0.22). Younger patients were diagnosed at a higher T stage (T3:27.9% vs 11.3%, \textit{p}< 0.001) and N (N0 vs N1-2, 47.6% vs 34.9%, \textit{p}=0.04), hence CS was higher among this group of age (IIIA: 24.2% vs 12.8%, \textit{p}=0.005). Pathological features, 80.3% presented high histological grade, positive margins (\textit{p}=0.008) and vascular permeation (\textit{p}=0.048) were identified in a younger population. Neoadjuvant chemotherapy (NAC) (24.2% vs 12.8%, \textit{p}=0.01), adjuvant chemotherapy (AC) (72.7% vs 46%, \textit{p}<0.001) and radiotherapy (RT) (56.8% vs 44%, \textit{p}=0.03) were less received in elderly patients, while mastectomy (51.7% vs 65.1%, \textit{p}=0.03) was mostly surgical technique used. There were no differences between locoregional (22.7% vs 32.6%, \textit{p}=0.07) and distant (31.1% vs 39%, \textit{p}=0.17) relapse rates. With a median follow-up of 8.8 years, at 5-year DFS, was better in younger patients (76% vs 60%, \textit{p}=0.02), but no difference was obtained in OS (80% vs 70%, \textit{p}=0.05). Chemotherapy showed to have an impact in a better DFS (HR:0.47, 95\%CI: 0.27-0.83) and OS (HR: 0.53, 95\%CI: 0.31-0.91, \textit{p}=0.02). Indeed, at 5-year OS was 89% for young patients with CT, 78% for elderly population with CT, 64% for ≥65y without CT and 54% for young without CT.

Conclusions: Despite the differences between characteristics of young and old patients, chemotherapy plays a critical role in DFS and OS in both populations. In elderly patients, chemotherapy should be considered in order to offer better outcomes.
Evaluation of menopausal status among breast cancer patients with chemotherapy-induced amenorrhea: A prospective study in China

Bailin Zhang¹, Rongshou Zheng¹, Qain Zhang¹ and Xiang Wang¹. ¹National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union of Medical College, Beijing, China.

Body: Background

Many pre-menopausal patients faced an unclear status of menstruation after chemotherapy, which makes it difficult for oncologists to determine the endocrinotherapy plan. This study aimed to establish a model that predicts and classifies the menstruation status of patients with CIA (chemotherapy-induced amenorrhea).

Methods

This is a hospital-based prospective study of female breast cancer patients. Inclusion criteria included patients diagnosed with breast cancer with a complete medical record of menses. Multivariate models were adjusted for established and potential confounding factors including age, serum concentration of estradiol (E2) and follicle-stimulating hormone (FSH), feeding, pregnancy, parity, abortions, and body mass index (BMI). The hazard ratios and 95% confidence intervals of different risk factors were estimated.

Results

From 2013 to 2016, 1,796 breast cancer patients were included in this study. Among them, 1,175 (65.42%) were pre-menopausal and 621 (34.58%) were post-menopause. The average age of natural menopause was 49.99 ± 3.24.

550 patients were included in CIA analysis, and 449 patients (81.64%) had CIA. As patients older than 60 years were defined as post-menopausal and low proportion of post-menopausal patients among people under 45 years old (4.83%), the target population of menopausal status estimation of patients focused on 1,142 patients aged 45 to 60 years old in this study. Age was found to be associated with menopausal status (OR: 1.856, 95% CI: 1.732-1.990). Other factors included, serum concentrations of E2 (OR: 0.976, 95% CI: 0.972-0.980) and FSH (OR: 1.060, 95% CI: 1.053-1.066), menarche age (OR: 1.074, 95% CI: 1.009-1.144) and number of abortions (OR: 0.829, 95% CI: 0.736-0.933). According to multivariate analysis, menopausal status was correlated with age, serum concentration of E2 and FSH. The model is: Logit (p) = -28.396+0.536Age-0.014E2+0.032FSH.

When $P$ is greater than 0.5, then the patient's menstrual state will be classified as post-menopausal. The areas under the ROC curve (AUC) were 0.9220, 0.8561 and 0.8769, respectively for the single factors of age, E2 and FSH, but the comprehensive model's AUC reached 0.9678. To examine the performance of this model, sensitivity analyses and model validation were conducted using external validation cohort (n=3,073), Leave-One-Out Cross Validation test, 10%, 20%, 30%- fold cross validation check, and Back substitution check. The results show that the sensitivities for different methods were about 85%, and the specificities were higher than 89%.

According to the estimation of National Center Cancer Registry of China and the result of a national-wide Chinese retrospective study, about 137.7 thousands newly diagnosed female breast cancer aged 45-60 each year. Among them, 30.72% was hormone receptor positive, and 41.4 thousands (14.85%) patients annually probably be influenced by chemotherapy and decisions regarding endocrine therapy must be concerned.

Conclusions

The discriminative model obtained from this study for predicting menstrual state is important for pre-menopausal breast cancer patients with CIA. This model has high levels of specificity and sensitivity. A large numbers of patients probably be benefited from this study.
Title: Tumor subtype concordance between breast and bone biopsies in bone only metastasis patients

Amanda M Parkes¹, Katherine K Clifton¹, Aydah Al Awadhi¹, Oluchi C Oke¹, Carla L Warneke¹, Jennifer K Litton¹ and Gabriel N Hortobagyi¹. ¹The University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Background:
Bone is the most common site of metastasis in metastatic breast cancer patients. Notably, bone biopsy is considered technically challenging with concerns regarding yield and reproducibility of immunohistochemistry technique. Our goal was to assess tumor subtype concordance between breast and bone biopsies done in patients with bone only metastases.

Methods:
We identified patients followed at MD Anderson Cancer Center for at least 6 months from 01/01/1997 to 12/31/2015 with bone as first site of metastasis. Breast and bone biopsy immunohistochemistry was used to categorize tumor subtype with hormone receptor positive (HR+) defined as ER or PR >1%. The following four tumor subtypes were identified: luminal A-like (HR+, HER2-), luminal B-like (HR+, HER2+), triple negative (HR-, HER2-), and HER positive (HR-, HER2+).

Results:
We identified 805 bone only metastasis patients with positive bone biopsies, 395 (49%) of which had hormone receptor and HER2 characterization available. Of these 395 patients, 293 (74%) were luminal A-like, 44 (11%) were luminal B-like, 51 (13%) were triple negative, and 7 (2%) were HER2 positive. Of these patients, we identified 281 patients with tumor subtype data available for both primary breast biopsy and bone metastasis biopsy, of which 237 (84%) were concordant, while 44 (16%) were discordant (Table 1).

Table 1. Concordance between breast and bone biopsies based on initial breast biopsy tumor subtype

<table>
<thead>
<tr>
<th>Breast Biopsy Tumor Subtype (n = 281)</th>
<th>Concordance with Bone Biopsy Tumor Subtype</th>
<th>Discordant Bone Biopsy Tumor Subtype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A-like, HR+ HER2- (225/80%)</td>
<td>Concordant: 199 (88%), Discordant: 26 (12%)</td>
<td>10 Luminal B-like, 16 Triple negative</td>
</tr>
<tr>
<td>Luminal B-like, HR+ HER2+ (33/12%)</td>
<td>Concordant: 19 (58%), Discordant: 14 (42%)</td>
<td>11 Luminal A-like, 2 Triple negative, 1 HER2 positive</td>
</tr>
<tr>
<td>Triple negative, HR- HER2- (20/7%)</td>
<td>Concordant: 16 (80%), Discordant: 4 (20%)</td>
<td>3 Luminal A-like, 1 Luminal B-like</td>
</tr>
<tr>
<td>HER2 positive, HR- HER2+ (3/1%)</td>
<td>Concordant: 3 (100%), Discordant: 0 (0%)</td>
<td>NA</td>
</tr>
</tbody>
</table>

Conclusions:
When available, bone biopsy tumor subtype had significant concordance with breast biopsy tumor subtype in this large study of bone only metastasis patients. Discordant tumor subtype results were more common in patients with luminal B-like tumor subtype on initial breast biopsy.
Title: Phase II study of the feasibility and safety of radium-223 dichloride in combination with hormonal therapy and denosumab for the treatment of patients with hormone receptor-positive breast cancer with bone-dominant metastasis

Rie K Tahara¹, Takeo Fujii¹, Babita Saigal¹, Nuhad K Ibrahim¹, Senthil Damodaran¹, Carlos H Barcenas¹, James L Murray¹, Beth A Chasen¹, Yu Shen¹, Diane D Liu¹, Gabriel N Hortobagyi¹, Debasish Tripathy¹ and Naoto T Ueno¹. ¹The University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Background
Radium-223 dichloride (Ra-223) is a therapeutic alpha particle-emitting radiopharmaceutical compound which have antitumor effect targeted on bone metastases. Alpha particles induces double strand DNA breaks and localized cytotoxic effect to cancer cells with limiting harm on normal tissues. We are conducting a phase II clinical trial of combination of Ra-223, hormonal therapy, and denosumab treatment in patients with hormone receptor (HR)-positive bone-dominant metastatic breast cancer (NCT02366130). In this preliminary analysis of the study, we aimed to evaluate the feasibility and safety of this combination therapy.

Methods
This single-center phase II study seeks to determine the efficacy and safety of Ra-223 in combination with hormonal therapy and denosumab. Major eligibility criteria include HR-positive breast cancer with bone and/or marrow predominant metastases. Patients with two or more visceral metastases were not eligible. There was no limit in the number of prior hormonal therapies in the metastatic setting. Patients received Ra-223 injection (55 kBq/kg intravenously) on day 1 of the study and then every 4 weeks thereafter for 6 cycles. Patients were also administered a single hormonal agent (i.e., tamoxifen, aromatase inhibitor, or fulvestrant at standard doses) daily and denosumab (120 mg subcutaneously) every 4 weeks. For this analysis, adverse events (AEs) were summarized using descriptive statistics.

Results
A total of 25 patients were enrolled and 22 were evaluable between March 2015 and December 2016. Median age was 58.5 years (range 31-79), and 59% of patients were postmenopausal. ECOG performance status was 0 in 16 patients (73%), and 1 in six patients (27%). HER2/neu was positive in only one patient. Four patients (18%) were de novo metastasis, no patients had visceral metastasis, and multiple bone metastases in 20 patients (91%) vs. focal metastasis in 2 (9%). Median time from diagnosis of bone metastasis was 4.8 months (range 0.5-96.6). Prior therapy for metastatic disease consisted of hormonal therapy in 50% of the patients (eight patients with one line and three patients with two lines), chemotherapy (9%), palbociclib (14%), radiation to bone metastasis (50%), and bone-supportive therapy (27% with zoledronic acid, 27% with denosumab). The median number of cycles of Ra-223 administered was 6 (range 4-6).

The median follow-up time was 4 months (range 2-8). There were no grade 3 or 4 AEs. Major non-hematological grade 1 and 2 AEs were bone pain (77%), fatigue (45%), nausea (36%), diarrhea (32%), AST/ALT elevation (23%), hot flashes (23%), and headache (18%). The most common hematological AEs were grade 1 or 2 neutropenia (23%), anemia (14%), and thrombocytopenia (18%). There was no treatment delay or discontinuation due to AEs.

Conclusion
Our results suggest that the addition of Ra-223 to hormonal therapy and denosumab is a feasible and safe combination therapy in patients with HR-positive breast cancer with bone-dominant metastasis. We continue to enroll patients in the phase II trial to evaluate the efficacy of the treatment.
Title: An open-label, multicenter phase 1b trial of radium-223 + paclitaxel in cancer patients with bone metastases: Safety results from the breast cancer patient subgroup

Sarah J Danson¹, Ruth Perets², Juanita Lopez³, Heikki Joensuu⁴, Avivit Peer², Samuel J Harris³, Fabricio Souza⁵, Bart Ploeger⁶, Kaline MC Pereira⁷ and Ravit Geva⁸. ¹Sheffield Experimental Cancer Medicine Centre, Weston Park Hospital, Sheffield, United Kingdom; ²Rambam Health Care Campus, Haifa, Israel; ³The Royal Marsden Hospital and The Institute of Cancer Research, Sutton, United Kingdom; ⁴Helsinki University Hospital, Helsinki, Finland; ⁵Bayer HealthCare Pharmaceuticals, Whippany, NJ; ⁶Bayer Pharma AG, Berlin, Germany; ⁷Bayer Pharma AG, São Paulo, Brazil and ⁸Tel Aviv Sourasky Medical Center, Tel Aviv, Israel.

Body: Background: Taxanes have an established role in treating breast cancer (BC), and combination with radium-223 (Ra-223) may be an option in patients (pts) with bone metastases. Both therapies impact hematologic parameters, but myelosuppression risk in combination is unknown. A phase 1b trial (NCT02442063) in cancer pts with bone metastases studied Ra-223+paclitaxel (PTX) safety and mode of interaction regarding myelosuppression; BC subgroup safety results are presented.

Methods: Eligible pts had a malignant solid tumor with ≥2 bone metastases and were PTX candidates. Treatment (tx) was 7 PTX cycles (90 mg/m²/wk IV per local standard of care, 3 wk on/1wk off) + 6 Ra-223 cycles (55 kBq/kg IV; 1 injection q4wk, starting at PTX cycle 2). Primary endpoint was % pts with neutropenia and thrombocytopenia during Ra-223+PTX (cycles 2, 3) vs PTX alone (cycle 1). A dose-exposure-response model describing time course of Ra-223+PTX–induced suppression of absolute neutrophil counts was used to evaluate Ra-223+PTX mode of interaction (additive or synergistic) in the total population.

Results: 15/22 enrolled pts were treated (total population); 7 had BC (BC subgroup). Baseline characteristics of the 2 groups were similar; ECOG PS was better in BC pts (Table). Fewer BC pts had prior taxane therapy (29% vs 53%), but rates of ≥3 prior chemotherapy regimens were similar (43% vs 47%). BC pts, vs total population, had slightly longer median tx duration for Ra-223 (6 vs 5.5 cycles) and PTX (7 vs 6 cycles), and more pts who completed 6 Ra-223 doses (57% vs 47%). Tx discontinuation related to disease progression in 29% of BC pts vs 33% in total population. Table shows TEAEs. In the BC subgroup, all 7 pts completed cycle 3 and Gr 3 neutropenia rates were 43% in cycle 2 and 14% in cycle 3, vs 29% in cycle 1; there was no Gr 4 neutropenia or Gr 3/4 thrombocytopenia. In the total population, 13 pts completing cycle 3 were in the pharmacodynamics analysis. Their Gr 3 neutropenia rates were 31% in cycle 2 and 8% in cycle 3, vs 23% in cycle 1; there was no Gr 4 neutropenia or Gr 3/4 thrombocytopenia. Myelosuppression model for the total population showed an additive effect of Ra-223 to PTX-induced neutropenia, with an additional 10% average decrease in absolute neutrophil count vs PTX alone. BC subgroup modeling was not feasible due to small sample size.

<table>
<thead>
<tr>
<th>Median age (range), y</th>
<th>Total Population n=15</th>
<th>BC Subgroup n=7</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tumor type, n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>61 (45-76)</td>
<td>58 (45-68)</td>
</tr>
<tr>
<td>Prostate</td>
<td>7 (47)</td>
<td>7 (100)</td>
</tr>
<tr>
<td>Bladder</td>
<td>4 (27)</td>
<td>1 (7)</td>
</tr>
<tr>
<td>Non-small cell lung</td>
<td>1 (7)</td>
<td>2 (13)</td>
</tr>
<tr>
<td>Other</td>
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<td></td>
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<tr>
<td><strong>ECOG score, n (%)</strong></td>
<td></td>
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</tr>
<tr>
<td>0</td>
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<td>5 (71)</td>
</tr>
<tr>
<td>1</td>
<td>8 (53)</td>
<td>1 (14)</td>
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<tr>
<td><strong>Prior taxane therapy, n (%)</strong></td>
<td></td>
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<tr>
<td></td>
<td>8 (53)</td>
<td>2 (29)</td>
</tr>
<tr>
<td>≥3 prior chemotherapy regimens, n (%)</td>
<td>7(47)</td>
<td>3(43)</td>
</tr>
<tr>
<td>-------------------------------------</td>
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<td>--------</td>
</tr>
<tr>
<td><strong>TEAEs, n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gr 3/4</td>
<td>9(60)</td>
<td>2(29)</td>
</tr>
<tr>
<td>Serious</td>
<td>6(40)</td>
<td>2(29)</td>
</tr>
<tr>
<td><strong>Gr 3/4 TEAEs, n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutrophil count decreased</td>
<td>6(40)</td>
<td>3(43)</td>
</tr>
<tr>
<td>White blood cell count decreased</td>
<td>4(27)</td>
<td>2(29)</td>
</tr>
</tbody>
</table>

TEAE = treatment-emergent adverse event.

*In >15% of patients.

**Conclusions:** Ra-223 was well tolerated when combined with PTX in pts with solid tumors and bone metastases. The BC subgroup vs total population had slightly higher hematologic AE rates, but fewer Gr 3/4 and serious TEAEs; more BC pts also completed study tx. The combination should be explored further in pts with bone metastases.
Somatic mutations, clinicopathologic characteristics, and survival in patients with untreated breast cancer with bone-only and non-bone sites of first metastasis

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Body: Background: Bone is the most common site of metastasis of breast cancer, and bone metastasis is associated with a high rate of skeletal-related events, all of which contribute to decreased quality of life and poor outcomes. Biological mechanisms of metastasis to bone may be unique, and identification of distinct signaling pathways and somatic mutations may provide biological insight into or rational targets for treatment of and prevention of bone metastasis. The aims of this study were to compare and contrast somatic mutations, clinicopathologic characteristics, and survival in breast cancer patients with bone only versus non-bone as first metastatic site.

Methods: Tumor samples were collected from 389 patients who had metastasis and untreated primary breast cancer. In each sample, 46 or 50 cancer-related genes were selectively amplified and analyzed for mutations by AmpliSeq Ion Torrent next-generation sequencing. We used Fisher’s exact test to identify somatic mutations associated with bone-only first metastasis and logistic regression models to identify differences in clinicopathologic characteristics, survival, and somatic mutations between patients with bone-only first metastasis and patients with first metastasis in non-bone sites only (“other-only first metastasis”).

Results: Among the 389 patients, the first metastasis was located in bone only in 72 patients (18.5%), non-bone sites only in 223 patients (57.3%), and both in 94 patients (24.2%). Of the cancer-related genes analyzed, the most commonly mutated were TP53 (N=103), PIK3CA (N=79), AKT (N=13), and PTEN (N=2). Compared to patients with other-only first metastasis, patients with bone-only first metastasis had higher rates of hormone-receptor-positive disease, non-triple-negative subtype, and low nuclear grade (grade 1 or 2) (all 3 comparisons, p<0.001); had a lower ratio of cases of invasive ductal carcinoma to cases of invasive lobular carcinoma (p=0.002); and tended to have a higher 5-year overall survival (OS) rate (78.2% [95% confidence interval (CI), 68.6%-89.0%] vs 55.0% [95% CI, 48.1%-62.9%]; p=0.051). However, in the subgroup of patients with TP53 mutation and in the subgroup of patients with PIK3CA mutation, OS did not differ between patients with bone-only and other-only first metastasis (p=0.49 and p=0.68; respectively). In univariate analysis, the rate of TP53 mutation tended to be lower in patients with bone-only first metastasis than in those with other-only first metastasis (15.3% vs 29.1%; p=0.051). In multivariate analysis, TP53 mutation was not significantly associated with site of first metastasis (p=0.54) but was significantly associated with hormone-receptor-negative disease (p<0.001).

Conclusions: We did not find associations between somatic mutations and bone-only first metastasis in patients with untreated breast cancer. Patients with bone-only first metastasis have longer OS than patients with other-only first metastasis. More comprehensive molecular analysis may be needed to further understand the factors associated with bone-only metastatic disease in breast cancer.
Incidence of skeletal-related events in patients suffering of bone metastasis from breast cancer treated with bisphosphonates or denosumab

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Body: Background
Bone is a frequent site of metastases and typically indicates a short-term prognosis in breast cancer patients. Bone metastasis can rarely be cured, but often it can still be treated to slow down its growth and to improve quality of life. The relative incidence of bone metastases in patients with advanced metastatic breast cancer is 65-75%. Bisphosphonates and the human monoclonal RANKL-antibody Denosumab are the most common medical treatment of bone metastasis. Therefore we investigated their effect on skeletal-related events (SRE) in our patient collective.

Materials and methods:
A retrospective analysis of 143 patients with histologically proven breast cancer and bone metastasis at the department of Gynaecology and Obstetrics at the University of Saarland (Germany) between October 2009 and October 2013. SREs were defined as a pathological fracture, a bone surgery, a spinal cord compression and a radiation of bone metastasis. Patients were distributed into 5 different groups according to the treatment received. Group 1: bisphosphonate (n=63), Group 2: denosumab (n=55), Group 3: bisphosphonate switch to denosumab (n=16), Group 4: denosumab switch to bisphosphonate (n=4), Group 5: no anti-resorptive therapy (n=5). Progression of the bone metastasis without skeletal-related event (SRE) was defined as progressive disease.

Results:
At the time of diagnosis of the breast cancer the average age was of 55,2±13,1 years. At the time of data collection n=53 patients were alive, n= 83 had died during follow up, and n= 7 were lost-of follow up. The median follow up from the first diagnosis of the primary breast cancer and the end of data collection was of 88,6 ± 78,5 month. In 103 (72%) patients bone metastases were detected secondarily. 83 patients recieved an anti-resorptive therapy with bisphosphonates (q4w), 75 patients were treated with denosumab (q4w).

In the overall collective, an SRE occured in 95 patients. 65.1% of Group 1(bisphosphonate) and 65.5% of Group 2 (densosumab) patients suffer from an SRE. In the non-treatment Group 2, 5 patients were diagnosed with SRE. The Switch groups showed the most frequent SREs (Group 3: 81,3%), (Group 4: 75,0%) the median duration of treatment prior to the first SRE was 12,33 month in Group 1, 1,5 month in group 2, 38,2 month in Group 3 and 4 month in group 4.

63 of 95 patients received a treatment modification after the first SRE. The time period between the first and second SRE was 31,8 month in Group 1, 10,38 month in group 2, 38,71 month in group 3, 10 month in group 4 and 3 month in group 5.

3,5% of all patients in the overall collective were diagnosed with an osteonecrosis of the Jaw.

Conclusion
This data suggests that the anti-resorptive therapy with densoumab is equal to the bisphosphonate therapy to prevent the first skeletal-related event in patients with bone metastasis of breast cancer. After the first SRE patients benefit significantly of an anti-resorptive therapy compared to no treatment. In our population the prevention of a second SRE in group 3 (bisphosphonate switch to denosumab)showed the best results.
Body: Background: The incidence of brain metastases (BM) in breast cancer patients is rising and has become a major clinical challenge. So far, the incidence of BM after modern neoadjuvant treatment is not clear.

Materials and Methods: In Geparquinto, patients with untreated HER2-positive breast cancer (n=615) received either lapatinib or trastuzumab, patients with HER2 negative breast cancer (n=1925) received bevacizumab in addition to an anthracycline and taxane-containing regimen and those not responding paclitaxel and everolimus (n=32). In Geparsixto, patients with HER2-positive tumors (n=273) received trastuzumab and lapatinib and patients with triple-negative tumors (n=315) received bevacizumab in addition to chemotherapy. We analyzed clinical factors associated with the occurrence of BM as first site of metastatic relapse after neoadjuvant treatment in both trials (n=3160).

Results: After a median follow-up of 61 months, 108 (3%) of a total of 3160 patients developed BM as first site of recurrence and 411 (13%) patients had distant metastases outside the brain. Brain metastases as first site of recurrence occurred later than other metastases (3-year-relapse free-rate 96.7% for patients who developed BM and 89.5% for patients who developed metastases outside the brain). Regarding subtypes of the primary tumor, 1% of luminal A (11/954), 2% of luminal B (7/381), 4% of HER2 positive (34/809) and 6% of triple-negative patients (56/1008) developed BM as first site of recurrence. In multivariate analysis, risk factors for the development of BM were larger tumor size (cT3-4; HR 1.9, 95%-CI 1.3-2.8, p=0.0022), node positive disease (HR 2.8, 95% CI 1.8-4.4, p<0.0001), no pCR after neoadjuvant chemotherapy (HR 2.7, 95% CI 1.6-4.7, p=0.0003) and HER2 positive (HR 3.8, 95% CI 1.9-7.8, p=0.0002) or triple-negative subtype (HR 8.1, 95% CI 4.2 – 15.8, p< 0.0001). Breast cancer subtype remained the most relevant risk factor for BM. Patients who developed BM were more often HER2 positive or triple-negative tumors compared with patients who developed metastases outside the brain (HER2 positive subtype 32 vs. 19%, triple-negative subtype 52 vs. 40%, p< 0.001).

Conclusion: Especially patients with HER2-positive and triple negative tumors are at risk of developing BM despite active systemic treatment. A better understanding of the underlying mechanisms is required in order to develop potential preventive strategies.
Title: Ado-trastuzumab emtansine (TDM-1) treatment and brain metastases in HER2 positive metastatic breast cancer patients: Final analysis of an Italian multicenter study

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Body: Background: Ado-trastuzumab emtansine (T-DM1) is a drug-antibody conjugate whose activity has been confirmed in HER2+ advanced breast cancer (BC) patients by the phase 3 EMILIA trial (Verma et al, NEJM 2012). Within the 991 patients enrolled in this trial, about 10% were affected with brain metastases (BM); in this subgroup, safety and efficacy of T-DM1 were confirmed although without any PFS improvement.

Patients and methods: In an Italian, multicenter, retrospective analysis involving 303 patients with advanced BC treated with T-DM1 (Fabi et al, Oncotarget 2017), we analyzed 87 patients with BM (BM-group). The study wanted to evaluate the efficacy of T-DM1 on BM; furthermore we compared BM-group with the remaining 216 patients without BM (nBM-group) in order to study outcome of disease. MRI was used as assessment imaging.

The number of extracranial metastatic sites in the BM-group and in the nBM-group was 1 for 10 (11.5%) and for 74 patients (34.3%), 2 for 23 (26.4%) and 93 (43%) patients, 3 for 25 (28.7%) and 38 (17.6%) and 4 or more for 29 (33%) and 11 (5%), respectively.

In the BM-group, 5 patients (5.7%) had received surgery alone as local treatment for brain metastases, 13 (14.9%) surgery plus stereotactic radio-surgery (SRS), 4 (4.7%) surgery plus whole-brain radiotherapy (WBRT), 23 (26.5%) SRS alone, 40 (45.9%) WBRT alone and 2 (2.3%) WBRT followed by SRS. Twenty-eight patients (32.9%) and 89 (42.4%) in the BM-group and nBM-group, respectively, received T-DM1 as second line, 24 (28.2%) and 49 (23.3%) as third line and 33 (38.8%) and 72 (34.3%) as fourth line. Mean number of cycles was 6 in both groups.

Results: Among BM-group, 53 patients (60.9%) were evaluable for response. Two (3.8%) obtained brain complete response, 14 (26.4%) partial response and 13 (24.5%) stable disease [brain disease control rate: 54.7%); 24 (45.3%) progressed during T-DM1.

Regarding extracranial metastases, overall response rate was 35.1% in the BM-group and 38.3% in the nBM-group; 6 months-clinical benefit was 50.6% and 52.3%, respectively. Median PFS was 7 months in the BM-group and 8 months in the nBM-group; when T-DM1 was given as second line, median PFS was 5 months in the BM-group and 11 months in nBM-group (p=0.01) while as third, line in which 76% of patients received lapatinib/capacitabine before TDM1, median PFS was 12 and 13 months (p=NS), respectively.

Conclusions: T-DM1 showed a good activity on BM in BC patients. A better outcome was shown in patients previously treated with lapatinib. The identification of clinical and biological prognostic factors could be needed to better select more responder patients with BM to T-DM1.
Title: Abemaciclib for the treatment of brain metastases secondary to hormone receptor positive breast cancer

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¹Centre Léon Bérard, Lyon, Cedex, France; ²University of Colorado Denver School of Medicine, Aurora, CO; ³Tennessee Oncology, Nashville, TN; ⁴Institut Curie Paris and Centre Eugene Marquis Renne, Paris, Cedex 05, France; ⁵Eli Lilly and Company, Indianapolis, IN and ⁶Institut Jules Bordet, Brussels, Belgium.

Body: Background: Although a lower percentage of patients with hormone receptor-positive (HR+) metastatic breast cancer (MBC) develop brain metastases when compared with triple negative and HER2+ MBC patients, there are no regulatory approved systemic agents for the treatment of HR+ breast cancer brain metastases, and this remains an unmet medical need. Standard local treatment options include surgery, stereotactic radiosurgery (SRS), and/or whole brain radiation therapy (WBRT). Abemaciclib, an oral selective CDK4 and 6 inhibitor administered on a continuous dosing schedule, has demonstrated clinical activity and an acceptable safety profile in heavily pre-treated HR+ MBC patients. Preclinically, abemaciclib crosses the blood-brain barrier, which is further supported clinically by detectable levels of abemaciclib similar to plasma levels in resected brain metastases in a subset of patients with HR+, HER2- MBC as previously reported for the current study. Together these data provide further rationale for evaluating abemaciclib in patients with brain metastases.

Methods: Study I3Y-MC-JPBO (NCT02308020) is an open-label, Phase 2, Simon 2-Stage trial evaluating the safety and efficacy of abemaciclib up to 200 mg BID in 4 cohorts of patients with brain metastases secondary to HR+ MBC, NSCLC, or melanoma. With regard to HR+ MBC, one cohort included HR+, HER2- patients, another one included HR+/HER2+ patients. All HR+ MBC patients enrolled to 1 of these 2 cohorts were required to have at least 1 measurable brain lesion. The primary objective was objective intracranial response rate as defined by Response Assessment in Neuro-Oncology brain metastases response criteria. Stage 1 was to enroll 23 evaluable patients per study part; if ≥2 respond to abemaciclib, 33 additional evaluable patients were to be enrolled to Stage 2. Secondary CNS objectives include best overall response, duration of response, and clinical benefit rate.

Results: For Stage 1 efficacy, in patients with HR+, HER2+ MBC futility was met. However, for HR+, HER2- patients, 2 confirmed, durable partial responses were observed and enrollment to Stage 2 is ongoing.

Conclusions: Previously, this study provided evidence that abemaciclib penetrates brain metastases in patients with HR+, HER2- MBC. The current results provide sufficient evidence of anti-tumor activity on brain metastases in patients with HR+, HER2- MBC to merit further exploration, but not for patients with HR+, HER2+ disease. Safety and tolerability results are similar to those previously reported for abemaciclib, with the majority of adverse events being gastrointestinal.
Clinical presentation and outcome of leptomeningeal metastasis in patients with breast cancer in relation to histology and tumor subtypes

Gaia Griguolo\textsuperscript{1,2}, Stephane Pouderoux\textsuperscript{3}, Maria Vittoria Dieci\textsuperscript{1,2}, William Jacot\textsuperscript{3}, Céline Bourgier\textsuperscript{4,5}, Federica Miglietta\textsuperscript{1,2}, Nelly Firmin\textsuperscript{3}, PierFranco Conte\textsuperscript{1,2}, Marie Viala\textsuperscript{3}, Valentina Guarnieri\textsuperscript{1,2} and Amélie Darlix\textsuperscript{3}.

\textsuperscript{1}Istituto Oncologico Veneto IRCCS, Padova, Italy; \textsuperscript{2}University of Padova, Padova, Italy; \textsuperscript{3}Institut Régional du Cancer de Montpellier (ICM), Montpellier, France; \textsuperscript{4}Institut Régional du Cancer de Montpellier (ICM), Montpellier, France and \textsuperscript{5}Institut de Recherche en Cancérologie de Montpellier (IRCM), INSERM U1194, Université de Montpellier, Institut Régional du Cancer de Montpellier (ICM), Montpellier, France.

Body: Background: Among solid tumors, breast cancer (BC) is one of the most common cause of leptomeningeal metastases. Leptomeningeal disease (LMD) typically carries a devastating prognosis; however, disease presentation and prognostic factors are still uncertain. The aim of this study was to characterize clinical features of LMD in relation to BC histology and subtypes.

Patients and Methods: 104 patients (pts) with LMD from BC diagnosed between 2002 and 2017 at two European institutions were included. LMD diagnosis was based on the presence of neoplastic cells on cerebrospinal fluid examination and/or radiological evidence of LMD. Patients’ characteristics and their associations with time from LMD to death or last follow up were evaluated by the Kaplan-Meier method, log-rank tests, and Cox proportional hazard models.

Results: Median age at LMD diagnosis was 56 yrs (range 26-75). Tumor histology (n=102) was ductal in 72 pts (70.6%), lobular in 22 (21.6%) and other histology in 8 (7.8%, including mixed ductal and lobular tumors). Tumor phenotype distribution was as follows: hormone receptor (HR)+/HER2- 54.8%, triple-negative (TN) 14.4%, HR+/HER2+ 12.5%, HR-/HER2+ 6.7% and unknown 11.5%. LMD diagnosis was cytologically proven (n=64, 62.7%) and/or radiologically proven (n=88, 85.4%). At time of LMD diagnosis, 63 pts (58.9%) had an ECOG performance status (PS) ≤ 2. 91 pts (87.5%) had extra-CNS disease localizations and 20 (18.7%) had a history of known BC brain metastasis (BM) (predating LMD diagnosis of more than 30 days). In lobular BC, LMD diagnosis was more frequently made in the absence of a known history of BM compared with ductal BC (95.5% vs 73.3%, Fisher test \( p = 0.036 \)). A majority of pts was treated with intrathecal (n=59, 55.1%) or systemic treatment (n=73, 68.2%) after LMD diagnosis, while only a minority underwent radiotherapy (n=28, 26.2%) or surgical derivation procedures (n=14, 13%). Median overall survival (OS) from LMD diagnosis was 3.2 months (95% CI, 1.9-4.4 months). No significant difference was observed across tumor phenotypes, with HER2+ subgroups experiencing better outcomes (median OS: 2.9, 1.6, 6.6 and 12.9 months in HR+/HER2-, TN, HR+/HER2+ and HR-/HER2+ subgroups; \( p = 0.54 \)). In univariate analysis, ECOG PS ≤ 2 at LMD diagnosis, intrathecal treatment and systemic treatment after LDM diagnosis were significantly associated with an improved OS (see table). Multivariate analysis showed that only ECOG PS ≤ 2 and systemic treatment after LMD diagnosis were independent factors associated with OS (see table). Updated results on an extended cohort of about 150 patients total will be presented at the meeting.

<table>
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<tr>
<th>Prognostic factors</th>
<th>Univariate</th>
<th>Multivariate</th>
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<td>Median OS in months (95%CI)</td>
<td>HR (95%CI)</td>
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<tr>
<td><strong>ECOG PS</strong></td>
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<td>≥3</td>
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<td><strong>Systemic treatment</strong></td>
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</tr>
<tr>
<td>No</td>
<td>1.0 (0.6-1.3)</td>
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</table>
Conclusions: LMD carries a dismal prognosis. The results of this series highlight that patient-related features and treatments (in particular the use of systemic treatment) contribute to modulate the prognosis of BC pts with LMD.
**Title:** Prognostic impact of initial treatment modality for brain metastasis in metastatic breast cancer

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**Body:** Background: The routine screening of brain metastasis (BM) is not recommended in patients with metastatic breast cancer (MBC) by guidelines. Due to survival improvement and increased use of magnetic resonance imaging (MRI) contribute the increasing of MBC BM incidence. The incidence of BM and survival from BM were various by molecular subtypes. The prognostic role of early detection of oligo BM and subsequent local treatment by subtypes is not clear. We aimed to compare BM free survival (BMFS) and BM overall survival (BMOS) by both treatment modalities and subtypes.

Methods: In the Yonsei Breast Cancer MBC Database, we identified 1252 MBC patients who were diagnosed from 2006 to 2016. Data of 358 MBC patients (127 HR+/HER2-, 80 HR+/HER2+, 64 HR-/HER2+, 87 HR-/HER2-) with BM were available for the analysis. BMFS was defined as the time from the diagnosis of MBC to the diagnosis of BM. MBC patients with initial BM were excluded from analysis of BMFS. BMOS was defined as the time from the diagnosis of BM to any cause of death. Treatment modalities were analyzed as three categories; whole brain radiotherapy (WBRT), stereotactic radiosurgery (SRS), and neurosurgery.

Results: Among the 1252 MBC patients, 47.7%, 14.7%, 13.1%, 17.6%, and 6.9% had HR+/HER2-, HR+/HER2+, HR-/HER2+, HR-/HER2-, and unknown subtypes, respectively. The incidence of initial BM were highest among patients with HR-/HER2- (14.5%) and were lowest with HR+/HER2- (5.2%)

<table>
<thead>
<tr>
<th>Initial metastatic sites</th>
<th>HR+/HER2-, n(%)</th>
<th>HR+/HER2+, n(%)</th>
<th>HR-/HER2+, n(%)</th>
<th>HR-/HER2-, n(%)</th>
<th>Unknown, n(%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>31 (5.2)</td>
<td>16 (8.7)</td>
<td>12 (7.3)</td>
<td>32 (14.5)</td>
<td>6 (6.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lung</td>
<td>197 (33)</td>
<td>72 (39.1)</td>
<td>62 (37.8)</td>
<td>99 (45)</td>
<td>33 (37.9)</td>
<td>0.036</td>
</tr>
<tr>
<td>Liver</td>
<td>137 (22.9)</td>
<td>46 (25)</td>
<td>52 (31.7)</td>
<td>56 (25.5)</td>
<td>16 (18.4)</td>
<td>0.122</td>
</tr>
<tr>
<td>Bone</td>
<td>403 (67.5)</td>
<td>109 (59.2)</td>
<td>66 (40.2)</td>
<td>90 (40.9)</td>
<td>48 (55.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Distant LN</td>
<td>176 (29.5)</td>
<td>74 (40.2)</td>
<td>60 (36.6)</td>
<td>93 (42.3)</td>
<td>22 (25.3)</td>
<td>0.011</td>
</tr>
<tr>
<td>Pleura</td>
<td>116 (19.4)</td>
<td>19 (10.3)</td>
<td>23 (14)</td>
<td>40 (18.2)</td>
<td>13 (14.9)</td>
<td>0.045</td>
</tr>
</tbody>
</table>

The median BMFS was 17.3 months (95% confidence interval (CI); 14.32-20.28) among the all patients with BM. The median BMFS was significantly shorter in the ER-/HER- subtypes (12 months, 95% CI; 20.73-31.67), and followed by ER-/HER2+ (14.8 months, 95% CI; 11.50-18.10), ER+/HER2+ (20.9 months, 95% CI; 16.88-24.92), and ER+/HER2- (26.2 months, 95% CI; 20.73-31.67). The median BMOS was significantly better in the SRS (16.8 months; 95% CI; 13.53-20.07) and surgery group (13.4 months, 95% CI; 7.19-19.61) compared with WBRT (6.9 months, 95% CI; 5.04-8.76) and no treatment (1.2 months, 95% CI; 0.32-2.08).

Conclusions: Incidence of initial BM and BMFS were significantly different by subtypes. Patients who were treated with SRS or neurosurgery showed better survival compared to WBRT. This findings support to consideration of screening of BM in HER2+ or triple negative subtypes for the early detection of oligo BM.
Title: Outcomes of central nervous system radiotherapy for metastatic breast cancer: The Royal Marsden experience 2000 - 2016

Gargi Kothari¹, Paolo B De Ieso², Kabir Mohammed¹ and Gillian M Ross¹. ¹The Royal Marsden NHS Foundation Trust, Sutton and London, United Kingdom and ²Alan Walker Cancer Centre, Darwin, Northern Territory, Australia.

Body: Background: Breast cancer (BC) is one of the most common malignancies affecting women. Brain metastases (BM) are frequently seen in BC, and can have devastating consequences with significant associated morbidity and mortality. Whole brain radiotherapy (WBRT) is commonly used to treat BM, with variable use of stereotactic radiotherapy (SRT). This study reports on the outcomes of BC patients with BM who received central nervous system (CNS) radiotherapy over a 17-year period at the Royal Marsden Hospital (RMH).

Methods: We included all BC patients who had WBRT with or without SRT for intra-parenchymal BM secondary to BC at RMH between 2000 and 2016 inclusive. Instances of meningeal involvement were excluded from analysis. Data collected included age, histological subtype, tumor grade, stage at original BC presentation, receptor status, date of BM diagnosis, number of metastases, size of largest BM, Eastern Cooperative Oncology Group (ECOG) score, presence of extra-cranial metastases (ECM), neurosurgery (NS) and stereotactic radiotherapy (SRT) details, and date of last follow up or death. Univariate and multivariate analyses were performed to analyze the effect of each variable on overall survival (OS) from date of BM.

Results: A total of 426 patients were included with a median age of 54 years at BM diagnosis and a median time to BM from BC diagnosis of 43 months. At diagnosis, 94% had invasive ductal carcinoma (IDC) and 70% had Grade 3 disease. Stage IV disease at original BC presentation was seen in 18% of patients. Estrogen receptor (ER+) was positive in 57% (n = 236), progesterone receptor (PR+) in 44% (n = 147), and HER2 (HER2+) in 44% (n = 166). Twenty-two percent (n = 89) were triple negative (TN). Median number of BM was 4 (range 1 – 205) and 20% (n = 72) of patients had only 1 BM. Average size of the largest lesion was 26 mm (range 1 – 75). The ECOG score was 0 – 1 in 61% of patients. Ten percent of patients (n = 44) underwent SRT and 10% (n = 43) underwent NS. Three hundred and eighty patients had died at the time of analysis. Median OS from date of BM was 6.4 months. On univariate analysis, age < 60 years at BC diagnosis (8.1 vs. 4.0 months, p = 0.0007) and BM diagnosis (8.0 vs. 5.6 months, p = 0.03), ECOG status 0-1 (9.6 vs. 4.0 months, p = <0.0001), ER+ (8.0 vs. 6.0 months, p = 0.0007), PR+ (7.6 vs. 6.9 months, p = 0.04), HER2+ (10.5 vs. 5.6 months, p < 0.0001), SRT (20.3 vs. 5.9 months, p < 0.0001) and NS (20.3 vs. 6.2 months, p < 0.0001) significantly predicted for improved OS. Triple negative status predicted for worse survival (5.6 months vs. 8.1 months, p < 0.0001). On multivariate analysis, ECOG status, ER+, HER2+, treatment with SRT and NS were independent predictors for OS.

Conclusions: This study confirms substantial heterogeneity of prognosis in patients with BM from BC, with significantly improved survival in patients selected for SRT or surgery. Further studies are required to optimize the role of CNS radiotherapy techniques such as SRT and hippocampal sparing WBRT in patients with a favorable prognosis.
**Title:** Preclinical characterization of neratinib in a blood-brain barrier co-culture model: Therapeutic implications for breast cancers with brain metastases

Tracey A Martin, Alshad S Lalani, Francesca Avogadri Connors, Richard P Bryce and Wen G Jiang. 1Cardiff University School of Medicine, Cardiff, United Kingdom and 2Puma Biotechnology, Inc, Los Angeles.

**Body:**

**Background:** Breast cancer patients who develop brain metastases have poor prognosis/short overall survival. Human epidermal growth factor receptor 2 (HER2) +ive breast cancer have an increased propensity for brain metastases. Currently, these metastases have limited therapeutic options. The endothelial cells forming the blood-brain barrier (BBB) are highly specialized to allow precise control over substances that enter/leave the brain via the tight junction. Targeted agents that are efficacious/able to penetrate the BBB are urgently needed. Neratinib is an orally available tyrosine kinase inhibitor that irreversibly binds and inhibits EGFR, HER2/HER4 receptor tyrosine kinases and has previously been shown to reduce the onset/delay symptomatic progression of CNS metastases in patients with advanced HER2-positive breast cancers with brain metastases (Awada 2016 JAMA Oncol; Freedman ASCO 2017 Ab 1005). Using a BBB model, this study aimed to determine if neratinib is able to pass through an endothelial barrier and demonstrate activity against human breast cancer cells lines. We also compared brain and vascular endothelial cells.

**Methods:** The BBB was represented by an in vitro transwell model using TY09 brain endothelial and HECV venous human cell lines in co-culture with a range of HER2+ive (MDA-MB-361;BT-474) or HER2-ive (MDA-MB-231;BT-549) human breast cancer cell lines. Effects on barrier function (trans-endothelial resistance/paracellular permeability) were assessed. Dose response effects on two brain endothelial cell lines (TY09/CMECD3) by neratinib were determined using ECIS (electric cell impedance sensing).

**Results:** Initially, we compared the effects of neratinib on a single brain endothelial cell line (TY09) in comparison to a venous endothelial cell line (HECV) in the presence/absence of EGF (20pg). We used temozolomide (TMZ) (40pmol) as a control treatment. Barrier function, assessed using trans-endothelial resistance and paracellular permeability revealed that neratinib had little effect on resistance over 1-72 hrs. When co-cultured with HER2+ive breast cancer cells, resistance was decreased at 1 hr which then returned to control levels (p<0.05). There was little change following treatment to the HER2-negative cells. A similar effect was also observed with TMZ. There was also a marked effect in HECV cells, but this was mostly observed in those treated with TMZ, regardless of HER2 status of the breast cancer cells in co-culture (p<0.04). Paracellular permeability was increased by 0.5 hr when using 10kDa (p<0.05) but not 40kDa dextran. Neratinib reduced the resistance of both TY09 and CMECD3 cells in a dose-dependent manner, with the same effect observed in cell attachment (p<0.01) and motility (0.05).

**Conclusions:** In our BBB co-culture model, neratinib was able to pass through the barrier created by both brain and venous endothelial cells and reduce the growth of HER2+ive breast cancer cells over 72 hrs. In addition, neratinib caused marked changes in barrier function of the brain endothelial cells with concurrent effects on cell behaviour. These data demonstrate that neratinib may be an attractive therapeutic for HER2-positive breast cancers with brain metastases.
Title: Efficacy of lapatinib and capecitabine combination therapy in brain metastases from HER-2 positive metastatic breast cancer: A systematic review and meta-analysis

Kyaw Z Thein¹, Myo H Zaw², Rachana Yendala¹, Henry P Igid¹, Chatree Chai-Adisaksopha³, Fred Hardwicke¹, Sanjay Awasthi¹ and Saba Radhi¹. ¹Texas Tech University Health Sciences Center, Lubbock, TX; ²The Brooklyn Hospital Center, New York, NY and ³McMaster University, Hamilton, ON, Canada.

Body: Background:
Brain metastases contribute to significant morbidity and mortality in breast cancer. Approximately one fourth of breast tumors overexpress the human epidermal growth factor receptor 2 (HER2) protein and are twice as likely to develop brain metastases. There are currently no systemic therapies approved. We undertook a systematic review and pooled analysis of trials to determine the efficacy of lapatinib and capecitabine combination therapy in brain metastases from HER-2 positive metastatic breast cancer (MBC).

Methods:
We performed a comprehensive literature search using MEDLINE, EMBASE databases, and meeting abstracts through December 31, 2016. Trials that utilized lapatinib and capecitabine combination therapy in brain metastases from HER-2 positive MBC were incorporated in the analysis. The pooled estimated rates were calculated using random effects model. Heterogeneity was assessed using $I^2$ statistic.

Results:
A total of 513 patients with brain metastases from HER-2 positive MBC from 6 trials and a subgroup of another 4 trials were included in our analysis. Lapatinib and capecitabine therapy was used as second-line treatment in 9 studies (n= 468) and as first-line treatment in the LANDSCAPE study (n= 45). Three studies were retrospective evaluations of randomized trials and the rest were phase 2 trials. CNS objective response rate (ORR) was 26% (95% CI: 19 – 33, $I^2$: 65.9%). Complete response (CR) rate was 1% (95% CI: 0 - 2, $I^2$: 0.0%) and partial response (PR) rate was noted at 24% (95% CI: 17 - 31, $I^2$: 66.1%). Stable disease (SD) occurred in 37% (95% CI: 29- 45, $I^2$: 66.6%) and progressive disease (PD) in 19% (95% CI: 12- 25, $I^2$: 66.5%). The first line LANDSCAPE study had the highest PR (49%) and ORR (53%) without a significant impact on CR rate; PD was 7%.

Conclusion:
Brain metastases in breast cancer is an area of urgent unmet need. Our meta-analysis showed that lapatinib/capecitabine therapy had some first line or second line activity in brain metastases from HER-2 positive MBC. Nevertheless, further randomized controlled trials are required in this patient population.
Leptomeningeal disease in ER+HER2- metastatic breast cancer patients: A review of the cases in a single institute over a 14-year period

Junichiro Watanabe¹, Koichi Mitsuya², Nakamasa Hayashi² and Yoko Nakasu². ¹Shizuoka Cancer Center, Shizuoka, Japan and ²Shizuoka Cancer Center.

Body: Background: Leptomeningeal disease (LMD) is a pattern of central nervous system (CNS) metastasis that occurs in metastatic breast cancer (MBC) patients (pts). Some reports have revealed that it occurs more frequently in pts with estrogen receptor-positive (ER+), HER2-MBC than in pts with other subtypes. However, in such ER+HER2-MBC pts, LMD mainly occurs in the terminal stage of the disease; thus, the details of LMD have not been well described.

Methods: We reviewed the medical records of ER+HER2-MBC pts who were treated from 2002 to present, with the aim of assessing the incidence, background and outcomes of LMD. Statistical analyses were performed using the chi-squared test, Kaplan-Meyer method, log-rank test and a multivariate COX regression analysis.

Results: We identified a total of 369 ER+HER2-MBC pts, and 102 (27.6%) developed CNS metastasis. LMD developed in 32 (8.7%) pts, with the median time to LMD of 778 days (95% confidence interval [CI] 335-1221; range 0-3757 days) from the diagnosis of MBC. In most cases (28, 87.5%), LMD was accompanied by bone metastasis, and 24 pts (75.0%) showed metastasis to the skull. Thirteen pts (40.6%) had accompanying brain metastasis (BM) at the diagnosis of LMD. The majority of the pts had symptoms (25, 78.1%), and their accompanying extra-CNS lesions showed progression (23, 71.9%). Palliative radiotherapy (RT) was introduced in 27 pts (84.4%), with 4 pts (12.5%) receiving whole CNS RT. The intrathecal injection of methotrexate was introduced to one patient. The median overall survival (OS) from the diagnosis of LMD was 104 days (95% CI 38-170); however, when limited to pts without BM (N = 19), the median OS was 146 days (95% CI 79-213). All of the pts died, and the causes of death were as follows: CNS lesion progression, n=10 (31.3%); cachexia, n=9 (28.1%); respiratory failure, n=8 (25.0%); hepatic failure, n=4 (12.5%) and infection, n=1 (3.1%). There was no significant relationship between the time to LMD and OS after the diagnosis of LMD (Spearman’s ρ=0.55, not significant). The multivariate analysis did not reveal any specific factors—such as the patient age, the presence of any symptom(s) at the diagnosis of LMD, the distribution of extra-CNS lesion(s) or the control of extra-CNS lesion(s)—that affected OS after the diagnosis of LMD.

As a control, 70 ER+HER2-MBC pts who developed BM without LMD (BM-only group) within the same observation period were analyzed. The median time to BM was 611 days (95% CI 404-818), and it did not differ from that of pts with LMD (LMD-group) to a statistically significant extent (P >0.1). The BM-only group showed superior OS after the diagnosis of their CNS lesions in comparison to LMD-group (median, 295 days and 104 days, respectively, P <0.001). At the diagnosis of the CNS lesion, the LMD-group showed a higher rate of CNS symptoms (P <0.01), a lower rate of liver metastasis (P <0.05), a higher rate of bone metastasis (P <0.05) and a higher rate of skull metastasis (P < 0.01).

Conclusion: Our retrospective analysis at a single institute revealed that the prognosis of LMD in pts with ER+HER2-MBC was still extremely poor. The data suggest that LMD is distinct from BM in terms of its pathology and response to therapy.
Body: Background
The incidence of brain metastases among women with metastatic breast cancer (MBC) ranges from 10 to 30% depending of breast cancer (BC) subtype. Inequities in the access to optimal treatment and shorter survival of BC by type of health care coverage were previously reported in an observational study in Brazil. The present analysis aims to analyze the impact of the type of health care coverage on survival outcomes of patients with MBC and brain involvement.

Methods
LACOG-0312 is a retrospective cohort study that enrolled patients with metastatic or locally advanced/recurrent unresectable BC diagnosed during 2012 in Brazil. Overall survival (OS) was defined as the time from the diagnosis of brain metastases and death from any cause. Comparisons were made using the Kaplan-Meier method based on the type of health care coverage (public vs. private) among patients who developed brain metastases. Cox regression analysis was performed for identification of independent prognostic factors associated with survival after brain metastases diagnosis.

Results
Among the 690 MBC patients included in the LACOG-0312 study, 145 (21%) were diagnosed with brain metastases. Of them, 94 (71.75%) were covered by the Brazilian public health care and 37 (28.25%) had private coverage. Baseline characteristics such as age at MBC diagnosis, stage IV at diagnosis and tumor subtypes were similar between both groups. Median time to develop brain metastases after diagnosis of MBC was 14 months in the whole population with no differences between public and private patients (13 vs. 17 months p=0.172). Median OS from the date of brain metastases diagnosis was similar for both groups: 10.0 months in private and 9.0 months in public health insured patients (HR 0.92 – 95%CI 0.55-1.51; p=0.729). In a multivariable analysis including type of health care coverage, only the triple negative BC subtype was associated with a worse survival post brain metastases diagnosis.

Conclusion
Our study indicates that health care coverage is not associated with survival outcomes in patients with MBC and brain metastases. Potential differences in the access to optimal care such as radiotherapy, surgery and systemic treatments may not play a significant role in the survival of theses patients possibly due to small clinical benefit of the current treatment options for brain metastases in breast cancer.
2017 San Antonio Breast Cancer Symposium

Publication Number: P2-01-01

Title: Trajectory patterns of circulating tumor cells (CTC) in chemotherapy-treated metastatic breast cancer (MBC) patients predict poor clinical outcomes: CALGB 40502 (Alliance)/NCCTG N063H study

Mark J Magbanua¹, Laura Hendrix², Terry Hyslop², William T Barry⁴, Eric P Winer⁴, Clifford Hudis³, Deborah Toppmeyer⁵, Harold Burnstein⁴, Misbah Qadir⁶, Cynthia Ma⁷, Janet H Scott¹, John W Park¹ and Hope S Rugo¹. ¹University of California San Francisco; ²Alliance Statistics and Data Center, Duke University School of Medicine; ³Memorial Sloan Kettering Cancer Center; ⁴Dana-Farber/Partners CancerCare; ⁵Rutgers Cancer Institute of New Jersey; ⁶UNC Lineberger Comprehensive Cancer Center and ⁷Washington University School of Medicine.

Body: Little is known about the dynamics of CTCs during treatment and its clinical significance. We examined the predictive utility of serial CTC analysis in ER+HER2- MBC patients (pts) treated with chemotherapy in the CALGB 40502/NCCTG N063H study, a randomized phase III trial of weekly paclitaxel compared to weekly nanoparticle albumin bound nab-paclitaxel or ixabepilone +/- bevacizumab as first-line therapy (ClinicalTrials.gov Identifier: NCT00785291, Support: U10CA180821, U10CA180882).

Methods: Of the 783 pts treated, 469 had ≥3 serial blood samples (including baseline) successfully analyzed for CTCs by CellSearch® and were included in this analysis (n=2,202). Samples with ≥5 CTCs per 7.5 mLs of blood were considered CTC+.

The prognostic and predictive performance of baseline CTCs (bCTC) and CTC status from baseline to cycle 2 (b2CTC) were compared to a novel latent mixture model classification based on trajectory of CTCs (tCTC). Akaike Information Criterion (AIC) was used to select the model (bCTC vs b2CTC vs tCTC) that best predicts overall survival (OS), progression-free survival (PFS), and time-to-treatment failure (TTF).

Results: 53% of the pts were CTC+ at baseline. b2CTC status changed in 36% of the pts, most of whom were CTC+CTC- (35%), and very few CTC-CTC+ (1%); the rest of the pts did not experience a change in b2CTC status (46% CTC-CTC- and 19% CTC+CTC+). Mixture model analysis revealed 4 groups of pts that show distinct tCTC patterns over the course of treatment: consistently very low/undetectable CTCs (tCTCneg, 56%), low (tCTClo, 24%), intermediate (tCTCmid, 15%), or high (tCTChi, 5%). bCTC, b2CTC, and tCTC were significantly correlated with tumor subtype (all p <0.0022) and presence of bone metastasis (all p <0.0001). Multivariate analysis showed that pts who were CTC+ at baseline, and those whose b2CTC status remained positive (CTC+CTC+) had significantly reduced OS, PFS and TTF.

<table>
<thead>
<tr>
<th>Models</th>
<th>OS HR (95% CI)</th>
<th>p-value</th>
<th>PFS HR (95% CI)</th>
<th>p-value</th>
<th>TTF HR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>bCTC (vs CTC-)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>⇒ CTC+</td>
<td>2.5(1.8-3.3)</td>
<td>&lt;0.0001</td>
<td>1.6(1.3-2.0)</td>
<td>&lt;0.0001</td>
<td>1.3(1.1-1.6)</td>
<td>0.0046</td>
</tr>
<tr>
<td>b2CTC (vs CTC+CTC-)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>⇒ CTC-CTC+</td>
<td>1.6(0.5-5.4)</td>
<td>0.4149</td>
<td>1.6(0.6-4.5)</td>
<td>0.3905</td>
<td>1.6(0.6-4.3)</td>
<td>0.3961</td>
</tr>
<tr>
<td>⇒ CTC+CTC+</td>
<td>2.7(1.9-3.8)</td>
<td>&lt;0.0001</td>
<td>1.8(1.4-2.5)</td>
<td>&lt;0.0001</td>
<td>1.8(1.3-2.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>⇒ CTC-CTC-</td>
<td>0.5(0.4-0.8)</td>
<td>0.0002</td>
<td>0.8(0.6-0.9)</td>
<td>0.0160</td>
<td>0.9(0.7-1.1)</td>
<td>0.2771</td>
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<tr>
<td>tCTC (vs tCTCneg)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>⇒ tCTClo</td>
<td>2.6(1.9-3.7)</td>
<td>&lt;0.0001</td>
<td>1.9(1.4-2.4)</td>
<td>&lt;0.0001</td>
<td>0.9(0.7-1.1)</td>
<td>0.0033</td>
</tr>
<tr>
<td>⇒ tCTCmid</td>
<td>5.3(3.6-8.0)</td>
<td>&lt;0.0001</td>
<td>2.5(1.8-3.4)</td>
<td>&lt;0.0001</td>
<td>1.8(1.4-2.5)</td>
<td>0.0001</td>
</tr>
<tr>
<td>⇒ tCTChi</td>
<td>10.8(6.1-19)</td>
<td>&lt;0.0001</td>
<td>3.0(1.8-5.0)</td>
<td>&lt;0.0001</td>
<td>2.3(1.4-3.7)</td>
<td>0.0009</td>
</tr>
</tbody>
</table>

CTC- (<5 CTCs per 7.5 mLs); CTC+ (≥5 CTCs per 7.5 mLs)

Pts with tCTClo, tCTCmid and tCTChi had significantly shorter OS, PFS and TTF compared to those with tCTCneg. After adjustment for potential confounders, AIC analysis revealed that the tCTC model best predicts OS and PFS, while b2CTC best predicts TTF.
<table>
<thead>
<tr>
<th>Models</th>
<th>OS</th>
<th>PFS</th>
<th>TTF</th>
</tr>
</thead>
<tbody>
<tr>
<td>bCTC</td>
<td>2432</td>
<td>4051</td>
<td>4199</td>
</tr>
<tr>
<td>b2CTC</td>
<td>2405</td>
<td>4038</td>
<td>4186</td>
</tr>
<tr>
<td>tCTC</td>
<td>2379</td>
<td>4026</td>
<td>4188</td>
</tr>
</tbody>
</table>

*The lowest AIC score indicates the best model.

**Conclusions:** Analysis of CTC trajectory patterns identified pts with poor outcome who could potentially benefit from more effective treatment. Validation in independent cohorts is warranted to confirm the findings in this study.
2017 San Antonio Breast Cancer Symposium

Publication Number: P2-01-02

Title: Heterogeneity and variability of human epidermal growth factor receptor 2 (HER2) expression on circulating tumor cells (CTC) in HER2 negative metastatic breast cancer patients treated with first line weekly paclitaxel and bevacizumab in a prospective cohort from the French Breast Cancer InterGroup Unicancer (UCBG): COMET study

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Body: Background: It has been reported in women with advanced estrogen-receptor (ER)-positive/(HER2)-negative breast cancer the acquisition of a HER2-positive CTC subpopulation during therapy (Jordan NV Nature 2016). The clinical significance of acquired HER2 heterogeneity during the evolution of metastatic breast cancer is unknown. We report here the analysis of HER2 status of CTC before and after one cycle of treatment in HER2 negative metastatic breast cancer patients treated with first line weekly paclitaxel and bevacizumab.

Patients & methods: The French cohort COMET is a prospective study including first line HER2 negative patients (pts) receiving weekly paclitaxel and bevacizumab according to EMA approved combination. The aim of this cohort is to evaluate clinical, biological and radiological parameters associated with pts outcome. We confirmed previously the outcome of patients with high CTC count at base line and after one cycle of treatment (Bidard et al, Lancet Oncol 2014). We present here the analysis on 203 pts evaluated for the expression of HER2 on CTC using the FDA cleared CellSearch method. The HER2 expression of CTCs (CB11 clone) was categorized (class 0–3) as described by Riethdorf et al.CCR2010.

Results: At base line, 144 out of 203 pts had at least one detectable CTC (71%), (median 4, and range 1-30,000). Among them, 104 (72%) had one or more HER2 positive CTC (1-21,484). In 25 patients with HER2 2+ primary tumor with FISH or CISH non amplified, the incidence of CTC HER2+ cases (13/25, 52%) was similar than in pts with HER2 0 or HER2 1+ (51%) primary tumor. In each case, 3 to 100% of detectable CTC could be HER2+ stained (median 50% of CTC). Only 12 cases (8% of all CTC cases) had 2+ HER2 staining score on CTC and none 3+. After one cycle of treatment, the number of pts with detectable CTC dropped to 64, including 42 with HER2+ CTC (65%). Out of these cases, 14 were 3+ or 2+ HER2 score (22% of CTC+ cases). This was a significant increase compared to baseline (8%) (p<0.001), including 6 cases with 100% of HER2+ CTC. To note, 7 patients without HER2+ CTC at baseline, had detectable HER2+ CTC after one cycle of treatment. With a median follow-up of 2 years, correlation of CTC variations with pts outcome is planned.

Conclusion: HER2 staining on CTC was heterogeneous with HER2 positive and negative subpopulations in the same patient with primary HER2 negative breast cancer. We observe a variability of HER2 CTC status with an increased intensity or appearance of immunostaining in few cases during treatment. We hypothesize that these phenotypes changes within patient-derived circulating tumor cells could contribute to progression of breast cancer and acquisition of drug resistance.
Improved prognostic information by serial monitoring of CTC enumeration and CTC-clusters from baseline to six months in patients with metastatic breast cancer scheduled for 1\textsuperscript{st} line systemic therapy

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**Body:** Background: Detection and enumeration of circulating tumor cells (CTCs) allows real time monitoring of disease evolution. In women with metastatic breast cancer (MBC), a CTC count of $\geq$5 CTCs is associated with decreased progression-free survival (PFS) and overall survival (OS). Serial sampling after therapy initiation has indicated that longitudinal CTC enumeration adds prognostic information, but data from long time sampling is sparse. The aim of this study was to evaluate if prospective longitudinal detection of CTC count and CTC clusters in women with newly diagnosed MBC can improve prognostication and monitoring of patients in the clinical setting.

**Methods:** Longitudinal blood samples were collected at baseline (BL) and after 1, 3 and 6 months in 156 women with MBC scheduled for 1\textsuperscript{st} line systemic therapy. CTC enumeration and cluster detection were performed by the CellSearch\textsuperscript{®} system in a prospective monitoring trial (NCT01322893). 115 patients had evaluable samples at all time-points. Primary endpoint was PFS and secondary endpoint was OS at BL in relation to CTC count and as landmark analyses during treatment. In addition, change in CTC count during therapy was compared to progressive disease (PD) versus non-PD. Structured clinical and radiological evaluation for PD was performed every 3\textsuperscript{rd} month.

**Results:** Seventy-nine (52\%) of 152 evaluable patients had $\geq$5 CTC and 14/79 patients had CTC-clusters ($\geq$3 clustered CTC) at BL. Median follow-up time was 25 (7-69) months. Patients with $\geq$5 CTCs had inferior PFS and OS in uni-(data not shown) and multivariable analysis (HR\textsubscript{PFS} 1.91 (1.26-2.91), $P=0.003$) (HR\textsubscript{OS} 3.57 (2.02-6.31), $P<0.001$) at BL. Presence of clusters at BL was prognostic for OS (HR\textsubscript{OS} 2.37 (1.25-4.51), $P=0.008$). Longitudinal landmark analysis of number of CTCs and presence of CTC clusters showed a time-dependent increase in HR during treatment for CTCs and CTC-clusters and predicted worse PFS and OS at all time-points. Stratifying patients based on CTC count and presence of clusters revealed four risk groups (0, 1-4, $\geq$5 CTC, $\geq$5 CTC + clusters) where patients with clusters had inferior PFS and OS at all time points. Change in CTC count from BL to 1 and 3 months, and from 3 to 6 months was significantly related to evaluation at 3 and 6 months (PD vs non-PD, $P=0.013$ (3 months), $P=0.016$ (6 months)) and change in CTC count from BL to 1, 3 and 6 months was also significantly predictive of both PFS and OS. Notably, survival was significantly inferior for patients with persistent CTC $\geq$5 during treatment.

**Discussion:** CTC is an independent prognostic factor for MBC patients scheduled for 1\textsuperscript{st} line systemic therapy. By longitudinal monitoring during treatment, the prognostic information by presence of $\geq$5 CTC and clusters increases over time and supports long time monitoring of patients. Importantly, detection of CTC-clusters identifies a subgroup of patients with dismal prognosis at all time-points indicating that CTC-clusters renders important clinical information. Change in CTC count during systemic therapy is related to outcome of evaluation and prognosis at all time-points.
Title: The whole transcriptional landscape of circulating tumor cells compared to metastases in stage IV breast cancer

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Body: Background: Metastatic breast cancer (MBC) and the circulating cells (CTCs) leading to macrometastasis are inherently different than primary breast cancer, evolving under the selection pressure of systemic therapy. A better understanding of the tumor biology of CTCs compared to metastasis may shed light on treatment opportunities.

Methods: We performed whole transcriptome sequencing (RNA Seq) on fresh metastatic tumor biopsies (mets), CTCs, and peripheral blood (PB) from 21 newly diagnosed MBC patients. CTCs were harvested using the ANGLE Parsortix to isolate cells based on size and deformability. Data were analyzed for differential expression, pathways, single nucleotide variants (SNV), fusions, intrinsic subtype, and a CTC-mets shared gene signature was validated using data from The Cancer Genome Atlas (TCGA). Detailed clinical-pathological and treatment data was evaluated.

Results: CTCs as a group showed much stronger gene expression of oncogenes, stem cell genes, keratins and mesenchymal markers than did mets from the same patients. Matched patient comparisons for 66 potentially clinically actionable genes for 8/9 pathways showed no significant difference in gene expression targets between CTCs and mets on ANOVA, although fold-change did vary. Eight SNVs in the ESR1 gene (n=5 patients) and 5 SNVs in the HER2 gene (n=2 patients) were shared between CTCs and distant metastases.

Differential gene expression analysis identified a signature of 8870 genes that were statistically significantly correlated between CTCs and mets (FDR adjusted p<0.05). Ingenuity pathway analysis was applied to the list of genes shared between CTCs and mets, with analysis of canonical pathways and upstream regulators revealing numerous oncogenes and breast cancer related genes. The top 50 genes of this CTC-mets shared signature were prognostic of worse overall survival in the TCGA breast cancer dataset (p<0.001), which included 817 patients with a median follow-up of 59.5 months. Second time-point data for n=5 patients with subsequent PB draws 6 months after baseline is currently pending. Intrinsic subtyping of mets by either NanoString assays or RNA Seq were not concordant with intrinsic subtyping of CTCs by RNA Seq.

Four of 21 CTC samples showed strong whole transcriptome RPKM correlation with PB (R²)>0.9, however, 3/21 CTC samples showed strong whole transcriptome RPKM correlation with mets (R²)>0.8. The remainder showed low correlation with both. Coverage was 91.4X for CTCs, 140.2X for mets and 138.5X for PB.

Conclusions: We present the transcriptomic landscape of CTCs with comparison to metastases and peripheral blood all acquired prior to treatment of newly diagnosed Stage IV breast cancer. Multiple genes, including oncogenes and stem cell genes, were found with higher expression in CTCs versus metastases. When focusing on 66 known potentially clinically actionable genes in breast cancer, CTCs did not show significantly different patterns of expression than mets in terms of up-regulation versus down-regulation compared to PB. RNA Seq of CTCs may be utilized to identify molecular alterations that are potentially clinically actionable.
**2017 San Antonio Breast Cancer Symposium**

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**Title:** Comprehensive analysis of genomic alterations in tumor tissue associated with presence of various subpopulations of circulating tumor cells (CTCs) in primary breast cancer

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**Body:** **Background:** CTCs play a major role in tumor dissemination and progression, and represent one of the key components of the metastatic cascade. The aim of this study was to identify signaling pathways associated with presence of CTCs in primary breast cancer (PBC) patients using a comprehensive genomics approach.

**Methods:** This translational study included 78 patients with PBC. CTCs were detected before surgery by quantitative RT-PCR assay for expression of epithelial (EP; CK19) or epithelial-mesenchymal transition (EMT) genes (TWIST1, SNAIL1, SLUG, ZEB1). Total DNA and RNA were extracted, in parallel, from fresh frozen primary tumor and the microRNA and mRNA expression profiles were obtained using Human microRNA Microarray v21.0 and SurePrint G3 Human Gene Expression v3 (Agilent Technologies). Next generation sequencing (NGS) was performed by Illumina Multiplex Sequencing using MiSeq Sequencing Reagent Kit V3.

**Results:** Mutations in \textit{BRCA1/2} genes in tumor tissue were more common in patients with epithelial CTCs (CTC_EP) compared to patients without epithelial CTCs in peripheral blood (23.5\% vs. 0\%, \(p = 0.02\)), while there were no mutations in specific genes associated with CTC with EMT phenotype (CTC_EMT). Further, we identified 90 genes and 7 miRs that were expressed at significantly different levels in tumors with presence of CTC_EP and 199 genes and 13 miRs specifically associated with CTC_EMT, compared to tumors with non-detectable CTCs. We also identified 39 overlapping genes and 7 miRs, that were expressed at significantly different levels in tumors with CTC_EP and/or CTC_EMT compared to tumors with non-detectable CTCs. Overlapping genes and miRs with highest different levels in expression were ATAD3A, TMEM201, DCPS, DOCK9-AS2, TRAF2 and miR-5195-3p, miR-188-5p, miR-6780a-5p, miR-6757-5p. Signalling pathways associated with these genomic alterations belong to several critically functional groups, such as immune response, signal transduction, cell proliferation, cell cycle progression, or apoptosis were significantly differentially based on CTCs status.

**Conclusions:** We identified for the first time various genomic alterations in primary tumor tissue of PBC associated with different CTCs subpopulations in peripheral blood. We hypothesize that these genomic alterations could play a role in tumor dissemination and progression and might lead to identification of new therapeutic targets.
Title: Similarities and differences in RNA profiles of circulating tumor cells in breast cancer subtypes: Do we have therapeutic options?

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Body: Background: The success or failure of anti-cancer therapies in breast cancer (BC) is only assessed retrospectively by the absence or presence of overt metastases during the post-operative follow-up period. Circulating Tumor Cells (CTCs), the precursor of metastatic disease, would be an ideal surrogate tissue to identify prognostic and predictive factors directly at primary diagnosis guiding to optimal individual therapeutic strategies for metastasis prevention. Since the prognosis in BC has been shown to be different in various BC subtypes, CTCs present in each subtype may, therefore, also represent distinct metabolic profiles for survival, metastatic spread and therapy resistance Using a multi-marker gene panel for the characterization of the heterogeneous CTC population, we here compared the genetic profiles of CTCs in triple negative BC (TNBC) patients (pts) with CTC characteristics in non-TNBC pts.

Methods: 2x5 ml blood of 43 TNBC pts and 51 non TNBC pts (11= HER2+/HR-, 40=HER2-/HR+) before and/or after neoadjuvant therapy were analyzed for CTCs applying positive immunomagnetic selection targeting EpCAM, EGFR and HER2 using the AdnaTest EMT-2/Stem Cell Select (QIAGEN Hannover GmbH, Germany). Subsequently, cDNA was gene specifically pre-amplified using TaqMan PreAmp Master Mix according to in house designed assays. Establishment of a 19 gene qPCR panel was performed for the markers PI3K, AKT2, ERCC1, Aurka, HER2, HER3, EGFR, ALK, AR (androgene receptor), BRCA1, c-KIT, c-MET, KRT5, mTOR, NOTCH1, PARP1, SRC1, CD45 (leucocyte control) and GAPDH (housekeeping gene) as well as an internal reference. The cutoff was calculated, taken the false positive rate in healthy donors into account and defined as Ct(cutoff)-Ct(sample)-[Ct(CD45cutoff)-Ct(CD45sample)].

Results: PI3K and mTOR as well as the resistance markers ERCC1 and AURKA were predominantly expressed in all BC subtypes, the latter especially after therapy. EGFR could not be detected in any BC subtype. In TNBC pts, all the different genes were expressed, probably representing the most heterogeneous CTC population. Interestingly, HER2/HER3+ CTCs were found before and after therapy in more than 20% of these pts and mTOR/PI3K expression were not reduced after therapy. In HER2+/HR- pts, ALK, AR, c-KIT, HER3, KRT5 were never detected whereas NOTCH, PARP1 and SRC1 seemed to be induced by therapy in about 30% of the pts. Notably, HER2+CTCs, initially detected in 50% of the pts, completely disappeared after therapy, most likely due to anti- HER2 targeted treatment which seemed to also markedly reduce initial PI3K/AKT/mTOR expression whereas resistant, AURKA+ CTCs were found in 40% of cases before and after therapy. The expression pattern in CTCs of HR+/HER2- pts was, although to a lower extend, similar to the profile detected in HER2+/HR- pts but compared better to ERCC1 based resistance induction in TNBC pts.

Conclusion: Although CTCs in TNBC pts and non-TNBC pts show different genetic profiles, the PI3K/AKT/mTOR pathway as well as resistance markers seem to be commonly expressed in CTCs of all BC subtypes. This knowledge about the individual target gene expression profile might efficiently help to predict a personalized targeted therapy for these pts in the future.
Isolation of viable CTCs from leukapheresis product with parsortix system enables subsequent culture

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**Body:** Background: Solid tumors are constantly releasing circulating tumor cells (CTCs) into the circulatory system. They are genotypically and phenotypically different from the primary tumor. Especially viable CTCs can be of high interest to obtain further therapeutically relevant information. Their extremely low frequency is one of the main limiting factors to use them for further characterization.

To overcome this challenge we tested to isolate viable CTCs from diagnostic leukapheresis (DLA) products obtained from breast cancer patients.

**Material/methods:** 16 DLA samples and matched peripheral blood (PB) samples of metastasized breast cancer patients were collected and CTC numbers were determined by CellSearch® analysis. Viable cells were enriched from DLA product with Parsortix system. Genomic DNA of single cultured CTCs was isolated and amplified by whole genome amplification. Array-based comparative genomic hybridization of single cells was performed to analyze for genomic aberrations. Resulting profiles were compared to genomic aberration profiles of CTCs isolated before in vitro culture.

**Results:** CTCs were detected in ten PB and matched DLA samples and in three more DLA samples whose corresponding blood samples were CTC-negative. CTC number per ml was increased by an average of 22.5 fold. Parsortix was optimized to enrich tumor cells from DLA product while keeping them best viable. An enrichment rate of 67.7% ± 11.3% and a harvesting rate of 65.3% ± 9.7% were determined with spiked MCF7 cells. We could grow viable CTCs from three out of eight CTC positive DLA samples from metastasized patients as determined by immune fluorescence analysis. The growing cells harbor genomic anomalies confirming their malignant origin. Most aberrations are widely identical to the aberrations detected in uncultured CTCs.

**Conclusions:** DLA provides greater numbers of viable CTCs which can be enriched with Parsortix system in order to enable their in vitro cultivation. This workflow will allow functional studies.
Title: Enumeration of heterogeneous circulating tumor cells (CTCs) using size-based method in early, and metastatic, breast cancer patients

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Body: Background
The detection of circulating tumor cells (CTCs) in peripheral blood is an independent predictor of the efficacy of systemic therapy, and also a prognostic marker for patients with metastatic breast cancer. One of the main methods to detect CTCs is CellSearch system, which uses immune-magnetic separation followed by immunocytochemistry. A microdevice (CTChip from ClearCell system) can capture and enumerate CTCs based on distinctive physiological differences (size and deformability) between cancer cells and blood cells. CTChip thus obtains a larger CTC yield than affinity-based separation, which enriches a particular subgroup of cells expressing EpCAM. In this study, we enumerate CTCs in peripheral blood from early and metastatic breast cancer patients using a size-based method.

Patients and methods
We examined blood samples from a total of 18 early and metastatic breast cancer patients, after obtaining written informed consent. Blood samples were taken in sodium EDTA tubes after discarding the first 1ml of blood from the syringe. Two ml blood samples were applied to CTChip (ClearCell system), and CTCs were eventually trapped in the microwells of the CTChip. Trapped cells were analyzed by immunocytochemistry with monoclonal antibodies specific for leukocytes (CD45) and epithelial cells (CK8/18), along with 4',6-diamidino-2-phenylindole (DAPI) for nuclei: CK8/18-positive, DAPI-positive and CD45-negative cells more than 10 µm in diameter were defined as CTCs. Eight patients were examined using both the CTChip and CellSearch system to compare the yield of CTCs.

Results
Of 18 patients, 6 were de novo stage IV, 6 were recurrent and 6 were early stage breast cancer patients. Of primary tumors, 8 were HER2- and ER and/or PR +, 6 were HER2-and ER- and PR-, 3 were HER2+ and ER and/or PR +, and one was HER2+ and ER- and PR-. Using CTChip, detected CTCs ranged from 3 - 107 cells/2 ml in all cases: 3 - 83 for early stage, 19 - 156 for stage IV and 21 - 146 for recurrent. The number of CTCs found in recurrent patients tended to be higher than in early stage patients. Size-based method using CTChip clearly showed high sensitivity compared with the CellSearch system, which detected CTCs in only 2 cases out of 8. In analysis by immunochemistry, we found CK-negative, CD45-negative and DAPI positive cells with larger diameter (>16 µm) than CK-positive CTCs in most patients, and the numbers were higher in stage IV (8.5 cells of median value) and recurrent (13 cells) patients than in early stage patients (1.5 cells). Our study suggested that CK-negative large cells might be CTCs with epithelial–mesenchymal transition (EMT).

Conclusion
This size-based technology enables us to capture CTCs regardless of EpCAM expression. Enumerated CTCs varied in size and positivity of CK8/18, suggesting the heterogeneity of CTCs. Further research, especially focusing on EMT will be crucial to understand the key mechanism of metastasis and drug resistance.
Title: Circulating tumor cells (CTCs) in the venous drainage of the breast in patients with primary breast cancer

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Body: Background:
CTCs are shed from tumors and circulate in the peripheral blood after passing through the drainage vein. Axillary lymph node dissection (ALND) provides access to the lateral thoracic vein which flows directly into the axillary vein. In this preliminary study, we evaluated the feasibility of detecting CTCs in the peripheral blood and in the lateral thoracic venous blood for breast cancer patients who underwent ALND.

Methods:
From June 2016 to March 2017, breast cancer patients who underwent ALND in our institute were eligible for this study. A peripheral blood sample, 10ml, was drawn just before the surgery or one day before the surgery. A lateral thoracic venous blood sample was taken from the resected breast just after resection. A blood sample of 0.2ml or more was necessary for CTC isolation. The CTCs in the peripheral blood before surgery (periCTC) and in the blood from the lateral thoracic vein of the resected breast (ltvCTC) were quantitatively examined by using a size-selective CTC isolation platform.

Results:
A total of 21 patients with median age 51 years (37-75) were enrolled to the study. Of the 21 patients, 38% were premenopausal, 52% had neoadjuvant chemotherapy. Fifty-seven percent were ER and/or PgR positive, 24% were HER2 positive. Fifty-seven percent were stage II disease and 43% were stage III. In 3 patients, we couldn't obtain sufficient blood samples from the lateral thoracic vein. Of the remaining 18 patients, we were able to obtain the median 0.5ml (0.2-2.0) blood samples from the lateral thoracic vein. CTCs were detected in peripheral blood in 15 patients (71%) and median periCTC count was 1 CTC/10ml (0-39). In lateral thoracic venous blood, CTCs were detected in all patients who had sufficient blood samples and the median ltv CTC count was 35.5 CTC/ml (2.5-370). In 5 of 6 patients whom CTCs in peripheral blood samples were not detected, CTCs could be detected in the blood samples from lateral thoracic vein.

Conclusion:
CTCs can be detected in the peripheral blood and in the blood from lateral thoracic vein in patients with localized breast cancer, and can be detected at a higher rate and at a higher concentration in the blood from lateral thoracic vein than in peripheral blood.
Title: Circulating CAF/CTC complexes and breast cancer metastasis

Body: Background: Metastatic disease in breast cancer (BC) is the leading cause of cancer-related mortality among women worldwide. Synergy between cancer cells and non-cancer cells of the tumor microenvironment (TME) are vital for disease progression. Cancer associated fibroblasts (CAFs) are the major cell type in the stroma of BC and are critical mediators of tumor progression and metastasis. Transport of circulating tumor cells (CTCs) and CTC clusters through the vasculature seeds metastasis and clinical and preclinical studies demonstrate that CTC clusters have a higher metastatic potential than individual CTCs. More recently, circulating cancer stem cells (cCSCs) have been implicated as more metastatic than non-CSC CTCs. In our lab, we have demonstrated that CAFs also circulate (cCAFs). We have observed cCAFs in peripheral blood from breast cancer patients and in murine models of breast cancer. Furthermore, we have observed that cCAFs are present in circulation as both individual cells and as well as in complexes with CTCs. Given the integral role of CAFs in BC metastasis, we hypothesize that cCAFs complex with CTCs/cCSCs to bolster BC metastasis.

Methods: cCAF/CTC clusters were identified and enumerated from peripheral blood of patients with BC, and associations with clinical features and disease outcomes were evaluated. Blood was collected by cardiac puncture from PyMT mice from 4 weeks through to the presence of metastases (10 weeks) and cCAF/CTC clusters enumerated. We co-injected CAFs with MCF-7 cells into NSG mice, blood collected by cardiac puncture, and cCAF/CTC clusters were enumerated. At time of final sacrifice, tumors were removed and assessed for presence of CSCs. Using our established model of cCAF/CTC clustering in vitro we interrogated cCAF/CTC complexing with both metastatic and poorly metastatic BC cells.

Results: Circulating cCAFs/CTCs clusters are significantly increased in the blood of patients with advanced stage BC and associate not only with severity of disease but also with poorer clinical outcomes. In the spontaneous PyMT mouse model, the appearance of circulating cCAF/CTC clusters increased significantly as tumors grew but prior to metastasis. We demonstrate that metastatic BC cells form clusters with CAFs in vitro while non-metastatic BC cells do not form complexes with CAFs in vitro. Enriching for stem cells from MCF7 mammospheres, resulted in CAF/CSC clusters in vitro. In mice that were co-injected with non-metastatic MCF7 cells and CAFs from a TNBC/Basal-like BC (CAF23) we observed disease metastasis, an enrichment for cancer stem cell (CSC)-like CTCs, and the presence of circulating cCAF/MCF7-CSC clusters.

Conclusions: Circulating clusters of CTCs and cCAFs are characteristic, and potentially causative, of BC metastasis. Observations of cCAF/CTC clusters from preclinical and clinical samples are corroborated by our determination that the ability of BC cells to form complexes with CAFs in vitro is related to the intrinsic metastatic ability of the breast cancer cells. Both in vitro and in circulation, the BC cells in cCAF/cBC clusters are CSCs, so cCAF/cCSC clusters. Disrupting the formation of cCAF/CTC complexes may be a potential strategy to reduce treat or prevent breast cancer metastasis.
Title: Dynamics of ctDNA changes during neoadjuvant chemotherapy in triple-negative breast cancer patients

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Body: Background:
Liquid biopsies to monitor response to treatment are a minimally invasive and highly attractive method for clinical application. Detection of ctDNA in plasma is now highly sensitive thanks to the use of novel highly sensitive and specific techniques such as ddPCR. In the present study we set out to analyze the utility of using ctDNA to monitor response to treatment in patients receiving standard neoadjuvant chemotherapy in triple negative breast cancer.

Methods:
Serial blood was collected from triple negative breast cancer patients participating in the Q-CROC-03 clinical trial (NCT01276899). The trial recruited triple negative breast cancer patients undergoing standard neoadjuvant chemotherapy. Paired biopsies were collected prior and at the end of treatment and serial biopsies collected throughout the study. Whole exome sequencing was performed on tissues collected and we identified mutated genes of interest. Cell free DNA (cfDNA) was extracted from 3 ml of plasma and 4-10 variants per patient were analyzed by ddPCR in serial plasma samples collected before and during treatment. Response was measured by evaluating residual cancer burden (RCB), and non-responders were RCBII-III, responders RCB0-I.

Results:
For the present analysis, we identified 60 variants in tumors from 12 patients (9 RCBII-III and 3 RCB0-I). Except for TP53, none of the genes were shared among the tumors. 20% of the variants were not detected in ctDNA at any time point and we did not find any correlation between cfDNA levels and tumor size or response to treatment. The average variant allele frequency (VAF) of all detected variants at baseline was higher in RCBII-III patients than in RCB0-I patients (7.0 vs 0.7 respectively). Interestingly, variants that were detected either only in the pre-chemo tumor or in the post-chemo tumor were frequently detected throughout neoadjuvant therapy, highlighting the ability of ctDNA to capture tumor heterogeneity. In almost all cases, we observed a dramatic decrease in ctDNA VAF after one cycle of chemotherapy, including 30% to non-detectable levels. By the 5th cycle of chemotherapy 97% of detected variants had decreased (average 95% decrease). This decrease in ctDNA VAF was independent of RCB score. In some RCBII-III cases, ctDNA VAF increased prior to surgery, reflecting residual tumor presence.

Conclusion:
ctDNA could be detected in plasma of all early TNBC patients undergoing neoadjuvant chemotherapy with the majority of variants detected in plasma collected at baseline prior to chemotherapy. Once treatment started, the abundance of ctDNA markedly decreased in plasma independently of tumor response. The effect of chemotherapy on levels of ctDNA needs further investigation.
Title: Assessment of an immune response panel of serum protein biomarkers for the non-invasive detection of breast cancer

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Body: Background: Breast cancer screening guidelines (ACS) recommend annual mammography for asymptomatic women ages 45 to 54 and once every two years for women age 55 and older. Women with suspicious screening mammograms are recommended for a diagnostic mammogram and may also undergo MRI or ultrasound. Ultimately, unresolved suspicious findings frequently result in the recommendation of a breast biopsy. Approximately 10% of suspicious diagnostic mammograms are recommended for breast biopsies and a large majority of these biopsies yield benign results. We conducted a screen for serum protein biomarkers and identified a novel panel for the non-invasive detection of breast cancer with the goal of developing a diagnostic test that can reduce the number of patients with benign pathology undergoing invasive biopsies.

Methods: Serum samples were collected at 4 US sites from women with suspicious diagnostic mammogram findings (primarily BI-RADS category 4 and breast composition b/c) undergoing biopsy for evaluation of a potential malignancy. Serum samples from 136 patients (87 benign pathology and 49 malignant pathology) were evaluated on the olink® Proteomics Immune Response Panel (92 analytes). Statistical screening methodologies, such as individual t-tests with control for false discovery, were used to identify markers with the potential to distinguish benign from malignant pathology. The candidate markers were further studied and combined using generalized linear modeling to develop potential diagnostic models.

Results: A 19-marker model resulted in an AUC of 0.94 with a sensitivity of 90% and a specificity of 80%. A 12-marker model resulted in an AUC of 0.93, yielding a sensitivity of 90% with a specificity of 77%.

Conclusions: This study reveals a novel panel of serum protein biomarkers that may allow for the non-invasive and sensitive detection of breast cancer in patients presenting with suspicious findings on mammography, thus reducing the need for invasive biopsies.
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Title: Circulating protein biomarker profile for inflammatory breast cancer using a multiplexed proximity extension assay

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Body: Background: Expression of cancer related genes and proteins in clinical specimens are the mainstay of personalized targeted therapy; however, a diagnostic signature for inflammatory breast cancer (IBC) remains elusive. In this study, we employed a blood-based, non-invasive, sensitive technology to map biomarkers in patients with IBC at the protein level. Proximity Extension Assay consists of a harmonious blending of immunoassay and PCR to amplify protein expression signal, thereby enabling multiplexing with small sample input (1 µl). Other multi-platform assays require a large amount of clinical material, multi-step sample processing and complicated data analysis.

Materials and methods: Serum samples (n=159) from patients with primary IBC (IBC, n=30), metastatic IBC (MIBC, n=54), locally advanced breast cancer (LABC, n=24) and metastatic breast cancer (MBC, n=27) were prospectively collected from subjects prior to starting a new therapy (treatment naive) or a new line of therapy between 2009 and 2012. Sera from 24 healthy normal donors (HD) were included in the analysis for comparison. The samples were analyzed using two panels: Proseek Multiplex Oncology II and Proseek Multiplex Inflammation I (Olink Proteomics, Uppsala, Sweden) for simultaneous detection of 92 human protein biomarkers in each panel. In the assay, each protein biomarker is detected by a matched pair of antibodies coupled to unique DNA-tags. Upon binding to the proteins, the correctly hybridized DNA-tags form an amplicon that can be measured by PCR. For initial analysis, sample populations were compared using the Mann-Whitney-U test.

Results: In comparison with HD sera, sera of breast cancer patients had 41 proteins from the oncology panel and 28 from the inflammation panel that were significantly higher, whereas 5 from the inflammation panel were significantly lower. From the inflammation panel, 11 proteins (PD-L1, IL-2, IL-7, IL-18, uPA, CCL4, CCL23, CXCL9, CXCL10, CXCL11 and TNF-alpha) showed significant differential expression between IBC and non-IBC derived samples (irrespective of metastatic status); for each marker, levels were higher in IBC than in non-IBC. In contrast, 9 proteins from the oncology panel (CRNN, CTSV, ERBB4, FR-gamma, ITGAV, MIA, PODXL, SCF and SEZ6L) were differentially expressed; however, each of these proteins was higher in non-IBC than in IBC. Among the aforementioned proteins, CCL4, IL-2, IL-7, PD-L1, TNF-alpha, uPA, CRNN, CTSV, FR-gamma, ITGAV, MIA, SCF and SEZ6L did not differentiate cancer and HD, but were uniquely characteristic of the IBC vs non-IBC comparison.

Conclusion: These preliminary data suggest that it is possible to distinguish between cancer patients and healthy normal donors, and also to delineate between IBC and non-IBC patients based on expression of serum proteins. Validation of this serum protein signature is planned in a larger patient cohort.
Title: Persistence of PIK3CA mutations detection in cell free tumor DNA as surrogate markers for hormonosensibility in patients with hormone receptor-positive breast cancer. The miRho clinical study

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Body: Background: HT resistance occurs in nearly all patients with mBC. The identification of early predictive biomarkers of HT failure could help tailoring monitoring or an early change in HT. Circulating biomarkers would allow, a global evaluation of all the metastatic sites without the need of invasive biopsies. Mutation in the phosphatidylinositol 3 phosphate kinase gene (PIK3CA) is one of the most frequent events in ER+ BC and has been involved in HT resistance. We evaluated the early predictive value of cell free DNA (cfDNA) PIK3CA detection in a population of first line HT BC patients.

Material and methods: 39 patients treated for an ER+/HER2- metastatic BC by first line HT in 2 French Comprehensive Cancer Centers were prospectively included in a dedicated clinical trial (NCT01612871) between June 2012 and January 2014. Serial blood sampling was performed before the initiation of HT (T0), 4 weeks (T1), 3 months (T3), 6 months (T6) and at tumor progression. Patients were followed until progression or end of the study (2 years follow-up). cfDNA was isolated from plasma using the QiaAmp circulating nucleic acid isolation kit (Qiagen). Mutation detection was performed using droplet digital PCR on a QX100™ system (Bio-Rad). The assay targeted wild type PIK3CA and mutations p.E542K, p.E545K in exon 9 and p.H1047R in exon 20. Target concentration was calculated as copies/reaction and cfDNA concentrations were reported as number of copies/mL of plasma. To assess the limit of detection of the three assays, isogenic reference DNA with known mutant allele frequency was used (Horizon Diagnostics). Based on confidence interval for Poisson parameter, a sample was considered positive if the average mutant copies detected was 4 copies and above per reaction.

Results: Median age of the population was 63 (range 40-86). HT was as follow: letrozole 32, tamoxifen 5, anastrozole 1 and exemestane 1 patients, respectively. Most patients (28, 71.8%) presented with non-measurable disease, precluding a relevant evaluation of predictive factors for response. Progression-free survival (PFS) was used instead as primary endpoint. Serum samples results were available for 37 and 35 patients at T0 and T1 respectively. PIK3CA mutations were present in 10 (27.8%) and 5 (14.3%) cases at T0 and T1 respectively. While presence of a cfDNA PIK3CA mutation in the T0 sample was not associated with PFS, the persistence of a detectable circulating mutation at T1 was highly significant of a worse PFS (40% vs. 76.7% at 1 year; p=0.0053).

Conclusions: In this dedicated clinical trial, 4-weeks persistence of cfDNA PIK3CA mutation appears highly correlated with PFS. Early identification of this mutated population could allow the evaluation of therapies targeting the PI3K/AKT/mTOR pathway in a selected population affected with an unfavorable prognosis. Dedicated studies and ancillary studies of such targeted therapies are warranted.
Title: Biomarker analysis by next generation circulating tumor DNA (ctDNA) sequencing in patients with advanced breast cancer

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Body: Background: Next-generation sequencing is of increasing interest to identify specific targets for both drug development and treatment. The study of metastatic cancer is complicated by lack of tissue and the potential for change in biology over treatment. We evaluated ctDNA in patients with advanced breast cancer to explore the relationship between specific DNA mutations and prognosis as well as therapeutic decision making.

Methods: Peripheral blood was collected in EDTA at the time of diagnosis of advanced disease. Samples were sent to Geneplus-Beijing for sequencing. Indexed Illumina libraries were prepared from germline and circulating DNA using the KAPA Library Preparation Kit; the capture probe was designed based on genomic regions selected with 1021 genes, covering the most frequently mutated genes and exons in solid tumors. Clinical characteristics, treatment and outcome data were collected. We analyzed progression free survival (PFS) from first-line therapy and overall survival (OS), endpoints were correlated with observed gene mutations.

Results: 54 patients were enrolled; 27 (50%) HER2+, 22 (41%) hormone receptor + (HR+)/HER2-, and 5 (9%) triple negative (TNBC). Median age was 48 (range 26-74). The median follow-up was 8 years (range 12-180 months). First-line therapy included chemotherapy with trastuzumab for HER2+ disease, chemotherapy with endocrine maintenance (17) or endocrine therapy alone (5) for HR+/HER2- disease, and chemotherapy for TNBC. Mutations were found in TP53, PIK3CA, PIK3CA 3140 A>G (p.H1047R), and ERBB (including ERBB1-4), at 40.7%, 35.2%, 20.4% and 25.9%, respectively. In univariate analysis, patients with tumor mutations in TP53 had a shorter OS (median 64 vs 121 months, p=0.006). The PIK3CA 3140 A>G mutation was more frequent in HER2+ (7/27, 25.9%) than HR+/HER2- (4/22 (18.2%) or TNBC (0/5), and was associated with shorter median PFS in HER2+ disease (mutant vs. wild type: 4 (range 2-9) vs. 8 (range 2-22) months, p=0.006). The frequency of ERBB mutation was similar in HER2- 7/27(25.9%) (p=0.707) or HER2+ 7/27(25.9%) disease (p=0.066); there was no significant impact on PFS in any subset. Multivariate analysis for HER2+ disease including age, ER, Ki67, TP53, PIK3CA, PIK3CA 3140 A>G and ERBB, demonstrated that the PIK3CA 3140 A>G mutation was the only factor associated with shorter PFS (p=0.025); further analysis by receiver operating characteristic (ROC) curve showed that the PIK3CA 3140 A>G mutation and the mutation in PIK3CA 3140 A>G and ERBB combination pathway had a large area under the curve (AUC), with AUC of 0.789, and 0.734 respectively.

Conclusions: Using NGS in ctDNA, we found that the PIK3CA 3140A>G mutation was more frequent in HER2+ disease, and was the only mutation associated with shorter PFS on multivariate analysis. The presence of a TP53 mutation was associated with worse OS. Evaluation of ctDNA is feasible in a general breast cancer population and has prognostic impact; further correlation of these findings with tumor samples is ongoing.
Title: Exosomal Del-1 as potent diagnostic marker for breast cancer: Prospective cohort study

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Body: Background: The authors recently reported exosomal Del-1 as a diagnostic marker for breast cancer Therefore, the current study aimed to confirm the diagnostic role of exosomal Del-1 in a prospective cohort by comparing the plasma exosomal Del-1 levels before and after curative surgery.

Patients and methods: To identify the optimal sampling time for the postoperative analysis, blood was serially collected at day 1, 3, 5, and 7 after surgery from 22 patients with breast cancer. Thereafter, 111 breast cancer patients who underwent curative surgery were prospectively enrolled to compare their exosomal Del-1 levels before and after surgery using an ELISA with both anti-Del-1 and anti-CD63 antibodies.

Results: Based on the population of 22 patients, the optimal sampling time was determined as post-operative day (POD) 3, at which point the exosomal Del-1 levels were all normalized and stabilized to 0.5 or less of the optical density (OD) value, as defined in a previous study of 21 patients with a high Del-1 level at diagnosis. Therefore, POD 3 or later was identified as the sampling time for the subsequent prospective cohort study (n=111). At diagnosis, 107 patients (96.4%) showed a high exosomal Del-1 level (OD value > 0.5), while 101 patients (94.6%) showed a normalized Del-1 level (≤0.5) after surgery, representing a significant difference (Mean OD value = 1.232 vs. 0.196; P < 0.00001). In the survival analysis, the clinical outcomes were compared with the Del-1 levels after surgery. During a mean follow-up of 35.9 (range, 11.9 – 70.6) months, 13 (11.7%) patients experienced relapses (5 loco-regional, 1 contralateral, and 7 distant): 4 out of 6 in the high group (>0.5), 3 out of 4 in the borderline group, and 6 out of 95 in the normalized group (≤0.4). The survival analysis also identified that a high postoperative Del-1 level was significantly associated with a worse progression-free survival adjusted to the clinicopathological characteristics, such as age, stage, histological grade, and subtype (hazard ratio [HR] = 24.0; 95% confidence interval [CI] = 3.5 – 163.9; P = 0.0011). Moreover, the HR was significantly higher when OD 0.4 was selected as the cut-off value.

Conclusion: The current prospective cohort study confirmed the normalization of exosomal Del-1 after curative surgery, indicating exosomal Del-1 as a potential potent diagnostic biomarker for breast cancer. Plus, since a high Del-1 level after surgery was also possibly linked with early relapse, this suggests exosomal Del-1 as a potential prognostic marker by identifying the existence of residual cancer, regardless of the risk factors.
Title: Validation of an autoantibody blood test for the detection of early breast cancer (BC), particularly hormone receptor positive BC

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Body: Background: Breast cancer (BC) remains the most common type of cancer in women, with an incidence of 1.6 million cases per year worldwide. Early detection through mammographic screening reduces BC mortality by ~20%, which while highly significant still leaves 80% of BC deaths unchanged. Furthermore, mammographic screening is only applicable to women between 50-70 years, in which only about one third of BCs occurs. There is an urgent need to identify a test, preferably a blood test showing high sensitivity and specificity for early BC across all ages of patients and all tumour types. Advances in understanding of the human autoantibody (AAB) response to early cancers has allowed us to harness this biological phenomenon for early cancer detection.

Aim: To identify a panel of tumor associated antigens (TAAs) which would detect AABs in the blood with high sensitivity and specificity for early BC: enabling cancer/normal discrimination.

Methods: Serum samples from 120 BC patients and matched controls were tested against a panel of 60 multiple TAAs using an optimised new multiplex microarray platform. A sub-group of 60 samples were also tested for AABs to estrogen receptor (ER). The selected TAAs were spotted onto a glass slide surface in an automated, highly reproducible system platform. If serum autoantibodies are present they bind to one or more of the TAA spots. Bound antibodies are detected with a fluorescent reporter and signal intensity measured using GenePix pro-6.

Data analysis: A Monte Carlo Simulation method was employed to define the best panel of the antigens with optimised cutoff points in each assay that would yield the highest sensitivity and specificity to discriminate BC patients from controls. AAB positivity in the BC group was analysed by tumor size (≤20mm versus >20mm), histological grade, lymph node status (positive or negative) and ER status (positive or negative).

Results: Using a panel of 12 TAAs, AABs were detected in pre-op blood of 34/60 (57%) primary BC patients compared to 9/59 controls (15%) (p=0.000003); one control sample data was unavailable. This gave a sensitivity of 57% and specificity of 85%. Median age of BC patients was 59yrs (20-81) versus 59yrs (28-81) in controls. There was no significant difference for AAB detection when compared to tumour size, grade, lymph node status or ER status. In the sub-group of 60 patients where ER antigen was measured using a panel of 8 TAAs AABs were detected in 20/29 BC patients compared to 2/30 controls (p=3.5e-7). This represents a sensitivity of 69% with a specificity of 93%.

Conclusions: These results confirmed our hypothesis that AABs can be detected in women of all ages with early BC. AABs were not related to tumour size, grade, lymph node status or ER status. If a panel of AAB assays can be validated it opens the possibility of a blood test for detection of early BC. Future direction of this research will be i) validation studies of a panel of AABs for detection of early BC, ii) detection of AABs at the earliest stages of carcinogenesis and iii) a panel of AABs which detect ER positive BC thereby enabling stratified chemoprevention in a high risk group combined with mammographic screening.
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Title: Breast cancer subtype distribution and circulating tumor DNA in response to neoadjuvant chemotherapy: Experiences from a preoperative cohort within SCAN-B

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Body: Introduction: Preoperative chemotherapy in early breast cancer increases the rate of breast preservation and provides prognostic information. In the case of residual disease, a change in subtypes may be observed. Sensitive and reproducible biomarkers predicting treatment response early during the treatment course are needed in order to better exploit the potential benefit of an individualized preoperative treatment.

Material and Methods: In an ongoing prospective study within the population-based SCAN-B project (NCT02306096), patients undergoing preoperative chemotherapy for early or locally recurrent breast cancer have been treated with iv Epirubicin and Cyclophosphamide q3w x 3 in sequence with either Docetaxel q3w x 3 or Paclitaxel q1w x 9 with a preoperative intent. HER2-positive cases also received HER2-directed treatment. At baseline, patients were staged using sentinel node biopsy for clinically node-negative patients and CT scan for cytologically confirmed node-positive cases. A clinical core needle biopsy as well as tissue from the surgical specimen was collected for determination of conventional biomarkers including ER, PgR, HER2 and Ki67. Tumor biopsies for biomolecule-extraction and RNA-sequencing were taken using ultrasound guidance and collected fresh in RNAlater at baseline, after 2 treatment cycles, as well as at surgery. Blood plasma samples were collected at baseline, after one-, three-, and six- 3w treatment cycles, and post-surgery. Using RNA-sequencing data, somatic mutations were identified in the tumor biopsies and personalized analyses for circulating tumor DNA (ctDNA) were performed. A pathological complete remission (pCR) was defined as the complete disappearance of invasive breast cancer in the breast and axilla at time of definitive surgery. Subtyping was performed using modified St Gallen criteria (2013).

Results: Thus far, 45 patients aged 24-74 years have been included, of which 34 (76 %) were clinical stage 2 and 11 (24%) were stage 3. The subtype distribution at baseline was five Luminal A-like (11 %), 21 Luminal B-like (HER2 negative) (47 %), 8 HER2-positive (18 %) and 11 Triple-negative (ductal) (24 %). The rates of pCR in 38 operated cases to date were 0/3 Luminal A-like, 3/19 Luminal B-like (HER2 negative), 2/8 HER2-positive, and 4/7 Triple-negative (overall 24 % pCR rate). One patient did not undergo surgery due to clinically progressive disease. In 25 cases with evaluable residual disease at surgery, there was a shift in the subtype in 13 (52 %), the majority of which represented a transition from Luminal B to Luminal A. No Triple-negative cases underwent a change in subtype during treatment. Results of the ctDNA analyses will be presented at the meeting.

Discussion: We have established an infrastructure allowing for an extensive evaluation of preoperative chemotherapy in early breast cancer. The goal is to develop methods to refine response-guided treatment in early breast cancer using molecular responses in the tumor as well as in the blood circulation. The patients continue to be prospectively monitored with iterative ctDNA analyses during follow-up.
Title: Circulating level of GP88/Progranulin is associated with clinical outcome and overall survival in stage 4 breast cancer patients

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Body: Monitoring of disease status in metastatic breast cancer (MBC) patients is a necessary step for an optimal management of patients during and post-therapy. Imaging technologies are the methods of choice in the standard of care to monitor therapy response and disease status in MBC patients. These methods are expensive, time-consuming and have limited sensitivity for real time monitoring. Measurements of circulating tumor markers CA15-3, CA125 and CEA have contributed, albeit with limitation, minimally invasive methods for MBC disease management. It is our hypothesis that measuring biomarkers involved in tumor biological processes may provide better evaluation of the disease state and thus aid real-time clinical management of MBC patients. Thus, addition of such new circulating disease biomarkers may improve the management of MBC patients. The 88kDa glycoprotein Progranulin (GP88/PGRN) fit these criteria. GP88/PGRN is expressed in tumor tissue and not in normal mammary tissue counterpart and secreted in the circulation of BC patients. Biological studies have established GP88/PGRN as a critical driver of BC cell proliferation, survival, invasiveness and drug resistance. Clinical studies have demonstrated that high tumor GP88/PGRN expression was prognostic for recurrence and that breast cancer patients had a statistically elevated GP88/PGRN serum level compared to healthy individuals. In the present study, we examined whether GP88/PGRN serum levels were elevated in MBC patients and whether GP88/PGRN circulating levels were correlated with patient clinical outcome and overall survival.

Under an IRB approved protocol at the University of Maryland Greenebaum Comprehensive Cancer Center, 101 stage 4 BC patients undergoing standard of care therapy and meeting the inclusion criteria were consented and enrolled. MBC patients’ demographics, clinical and disease characteristics and therapies were collected as part of the study. Blood samples were collected from each patient at specific times at follow-up visits during and post-therapy. The prepared serum was stored at -80C until tested for GP88 using a GP88 enzyme linked immunoassay developed in our laboratory. Statistical analysis using Kaplan-Meier functions established whether there was a correlation between GP88/PGRN serum level and overall survival in MBC patients. MBC patients with distinct survival characteristics (P=0.0002) could be stratified based on their circulating GP88/PGRN levels. Analysis of this association was carried out in MBC patients based on their age, race, tumor characteristics, receptor status and metastatic burden (number and sites of metastasis) and will be reported. We conclude that circulating levels of GP88/PGRN in MBC patients are correlated with overall survival and that monitoring circulating GP88/PGRN levels would provide additional information and valuable insight into real-time MBC disease status.

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Title: Circulating HER2 extracellular domain (ECD) predicts a poor prognosis for metastatic breast cancer

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Body: Background: Amplification or overexpression of HER2 has been shown to play an important role in approximately 30% of breast cancers and is strongly associated with increased recurrence and a worse prognosis. The HER2 extracellular domain (ECD) may be cleaved and shed from the surface of breast cancer cells and serum ECD can be detected by enzyme-linked ELISA.

Methods: Two hundred and seven metastatic breast cancer patients from March 2009 to July 2011 were involved in this prospective study at Zhejiang Cancer Hospital. In this study, we explored the relationship between circulating HER2 ECD and clinical pathological characteristics, included HER2 status. Then we can predict the outcome of metastatic breast cancer patients and determine the predictive value as a valuable prognostic factors. Serum HER2 ECD levels were measured by ELISA. Tissue HER2 was determined by IHC and FISH in tumor samples, respectively.

Results: For the metastatic breast cancer patients, the percentage of cases with high concentrations (≥15 ng/ml) of serum HER2 ECD is 39.1% (43/110) in HER2-positive cases and 23.4% (22/94) in HER2-negative cases (P=0.017). High serum HER2 ECD levels (≥15 ng/ml) were significantly associated with elevated serum CEA (52.1% v 21.5%, OR 3.978, 95%CI 2.138-7.401, P=0.000), CA125 (48.5% v 23.5%, OR 3.064, 95%CI 1.650-5.691, P=0.000), CA153 (53.2% v 17.1%, OR 5.48, 95%CI 2.897-10.366, P=0.000), LDH (53.3% v 23.1%, OR 3.798, 95%CI 2.011-7.173, P=0.000) and AKP (51.2% v 26.5%, OR 2.911, 95%CI 1.442-5.879, P=0.002) in metastatic breast cancer patients. There were still statistically significant correlation between increased serum HER2 ECD levels and the site of relapse, visceral involved (37.9% v 14.8%, OR 3.511, 95%CI 1.548-7.961, P=0.002), liver(42.7% v 24.0%, OR 2.358, 95%CI 1.294-4.297, P=0.005), brain (50.0% v 26.7%, OR 2.744, 95%CI 1.397-5.391, P=0.003), respectively.

Conclusions: Monitoring the circulating levels of the HER2 ECD in patients with metastatic breast cancer provides a real-time assessment of tumor burden and indicates poor prognosis, and may provide important information for reassessment of HER2 in HER2-negative metastatic breast cancer.
Title: Cell free DNA analysis identifies actionable ERBB2 amplifications in patients with HER2 negative breast cancer

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Body: Identification of ERBB2 (HER2) overexpression in metastatic breast cancer informs utilization of HER2 targeted therapy. The NCCN recommends HER2 expression re-evaluation at the first disease recurrence in patients with negative or equivocal tissue status given results discrepancies due to inadequate tissue biopsy, tumoral heterogeneity, biopsy technique or fixation as well as discordance in ERBB2 (HER2) expression between primary and metastatic lesions. We examined the incidence of ERBB2 (HER2) negative to positive “flips” (e.g. to ERBB2-amplified in plasma) in a cohort of patients who underwent a blood-based cell-free DNA (cfDNA) assay at a CLIA-certified/CAP-accredited/NYSDOH-approved molecular diagnostic laboratory. Laboratory database was queried for samples from patients with a breast cancer diagnosis. The query was filtered to ensure patients with multiple cfDNA timepoints were counted only once. Patients without a pathology report submitted at any cfDNA collection timepoint or the pathology report did not include ERBB2 (HER2) status, results were inconclusive or quantity not sufficient were excluded. Between March 2014 and April 2017, 1,853 unique patients were identified with reported ERBB2 (HER2) status. For patients with more than one cfDNA timepoint collected (N=349; 18.8%), the earliest pathology report was referenced. 1,386 patient tumor samples were negative for HER2 overexpression (74.8%), 325 (17.5%) were positive, and 142 (7.7%) were equivocal. Twenty-nine of the 1,386 patients with reported tumor negative HER2 status had amplification on subsequent cfDNA analysis (2.1%).

All 29 patients were female. Most patients (N=21) had a single cfDNA timepoint collected. Median age at cfDNA blood draw was 58 years (range 28–68). Median length of time between reported tissue negative status and cfDNA blood draw was 405 days (range 21–4,060). Median plasma ERBB2 copy number was 2.44 (greater than 50th-centile per laboratory data) (range 2.15–16.5).

Clinical follow-up was obtained for 19 patients (65%). Nine patients were lost to follow-up or succumbed to disease prior to initiation of a new therapeutic regimen. One patient was known HER2 positive prior to receipt of the cfDNA results. In the remaining nine patients, six initiated targeted HER2 therapy following receipt of the cfDNA results, with five of six (83%) demonstrating a clinical response. In one patient with known ER/PR positive, HER2 negative disease, progressing through multiple lines of therapy, addition of trastuzumab and pertuzumab to her paclitaxel regimen following identification of the cfDNA ERBB2 amplification resulted in a significant reduction in CEA levels (238 to 37.9 ng/mL) by week five. In a second patient, following identification of the cfDNA ERBB2 amplification, she was treated with trastuzumab and pertuzumab along with docetaxel and had a dramatic response. She continues on trastuzumab and pertuzumab alone. Although a modest sample size, this is the second cfDNA series demonstrating that ERBB2 (HER2) status may flip from negative to positive upon recurrence or metastasis, and that targeting plasma-detected ERBB2 amplification with anti-HER2 has clinical benefit. cfDNA is a viable alternative to tissue rebiopsy in this patient population.
**Title:** Utilization of cell-free circulating tumor DNA for management of breast cancer: Practices in academic and community oncology

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**Body:** Background: Next-generation sequencing (NGS) of cell-free DNA (cfDNA) is increasingly being utilized to assess somatic genomic alterations in patients with breast cancer. We investigated the clinical use of such testing in breast cancer care in a major healthcare system with both academic and community-based practices. We also explored the observed genomic landscape in the analyzed patient cohort and whether treatment plans were modified based on the results.

Methods: A retrospective review of cfDNA NGS results (Guardant360) ordered at the University of Pittsburgh Medical Center for patients with breast cancer from 7/2015-3/2017 was performed. Test ordering patterns, the landscape of genomic alterations identified, and clinical use of select results were assessed.

Results: During this period 95 samples were submitted, 73 (77%) ordered by academic center providers and 22 (25%) from community providers. Alterations were detected in 88 samples (93%) with a median of 3 alterations per test. Five patients had serial samples ordered assessing dynamic cfDNA across clinical treatment and progressions, leaving 84 unique patients in the dataset. The average patient age was 57, and 95% of patients were female. Patients were most often observed to have alterations in TP53 (51%), PIK3CA (44%), and ESR1 (26%). Additional clinical data were collected for 48 patients with mutations or amplifications in PIK3CA, ESR1, and/or ERBB2 (HER2) to assess for clinical use of genomic information. Results were used to change clinical care in 13 (27%) of these cases. Community providers were more likely to use genomic results to guide clinical management in these cases (9/16, 56%) than academic providers (4/32, 12.5%), p=0.001. Of this patient subset, those with tests ordered by an academic provider had more lines of prior therapy at the time of testing vs. those in the community (average 5.9 vs 3.4 respectively, p=0.019).

Conclusions: CfDNA NGS analysis for somatic genomic alterations in breast cancer is being ordered clinically by both academic and community practices within this healthcare system. Results for a subset of clinically annotated patients were acted on more frequently by community-based ordering providers, which may be related to patients tested at academic sites having had more lines of prior treatment.
Circulating tumor DNA predicts clinical outcome in early stage triple negative breast cancer

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Background- Triple negative breast cancer (TNBC) is the most aggressive subtype of breast cancer as these patients have the highest risk of recurrence and death. Only 35% of TNBC patients achieve a pathologic complete response (pCR) following neoadjuvant chemotherapy. Patients who do not achieve pCR have a 27% risk of distant recurrence and ultimate death at 3 years compared to 9% for pCR. Unidentified micrometastases are responsible for ultimate overt progression and death. Developing strategies to identify patients with minimal residual disease following curative treatment is an unmet need. Circulating tumor DNA (ctDNA) can characterize and monitor advanced cancers. In this study, we sought to assess if ctDNA can predict clinical outcome in TNBC.

Methods- Biospecimens were obtained from patients with stages II and III TNBC enrolled on a neoadjuvant trial (NCT02124902). Patients have a research biopsy and plasma for ctDNA collected at baseline, cycle 1 day 3, definitive surgery for those with residual disease, and at recurrence for those who relapse. Plasma for ctDNA is also collected every 6 months for 5 years after treatment. Patients receive docetaxel and carboplatin every 3 weeks X 6 cycles. Surgery is 3-5 weeks after chemotherapy. Six patients' serial tumor samples and germline DNA were studied by whole exome sequencing. The median sequencing depth was 90.13x. Sequencing was performed on samples with high cellularity (≥50%). All 6 patients also had serial ctDNA analyzed using Swift Biosciences Accel-Amplicon™ 56G Oncology Panel v2. After identifying somatic mutations in each breast tumor series, we determined the subset of mutations that intersected with the regions targeted by the Swift 56 gene panel. We then evaluated whether corresponding mutations could be detected in ctDNA, and if ctDNA predicted clinical outcome.

Results- Four of the 6 patients were non-pCR with residual disease following chemotherapy. We identified 627 somatic variants by exome analysis that were called by at least two somatic variant callers and passed additional quality filtering steps. Of these, 10 variants overlapped with the Swift panel. TP53 variants were identified in all 6 patients' tumor tissue samples. At least one TP53 variant was identified in 4 patients' baseline pre-chemotherapy ctDNA samples. Both pCR patients had either no detectable ctDNA TP53 mutations (NTN007-ref. in baseline tumor tissue was 19.58% variant allele frequency [VAF]); or clearance of ctDNA following chemotherapy from 4.45% VAF at baseline to 0.06% following chemotherapy (NTN004-ref. in baseline tumor tissue 37.34% VAF). Three non-pCR patients had persistent TP53 mutations in ctDNA during the treatment course. One non-pCR patient did not have detectable mutations in ctDNA. The only patient with recurrent disease whose ctDNA TP53 mutation persisted during the treatment course (baseline VAF-1.65%, cycle 1 day 3-0.78%, definitive surgery-0.09%), was found to have a higher ctDNA VAF at recurrence (29.55%).

Conclusion- In this pilot study, mutation tracking by ctDNA is sensitive and distinguishes pCR from non-pCR in TNBC patients receiving neoadjuvant chemotherapy. ctDNA also identifies recurrence following curative therapy. Evaluating ctDNA as a biomarker of outcome in TNBC is warranted.
Title: Elevated serum RAS p21 is an independent prognostic factor in metastatic breast cancer

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Body: Background
An important component of the RAS signalling pathway, the RAS p21 oncogene, is frequently hyperactivated in breast cancer. Its expression in tumor tissue has been linked to poor clinical outcome. This study was designed to evaluate the clinical relevance of RAS p21 levels in peripheral blood in a large cohort of metastatic breast cancer patients.

Methods
251 patients with metastatic breast cancer were enrolled in this prospective, multicentre, non-randomized study conducted on behalf of the DETECT Study Group (Current Controlled Trials ISRCTN59722891). Blood samples were collected before start of first-line or later-line treatment. RAS p21 was determined using a sandwich-type ELISA immunoassay. For the determination of the cutoff, blood samples from age-matched healthy controls were analyzed. A value above 452 pg/ml was regarded as elevated (mean + 2 x SD). In the univariate survival analysis, two other cutoffs were considered as well (50th and 75th percentile of patients, i.e. 229 pg/ml and 320 pg/ml). Circulating tumor cells (CTCs) were detected using the CellSearch system.

Results
29 of 251 (12%) patients had RAS p21 levels above the cut-off level of 452 pg/ml. Clinical-pathological parameters, such as hormone receptor and HER2 status, line of therapy and CTC status, did not correlate with RAS p21 levels.

Patients' characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>RAS p21 elevated n (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>251</td>
<td>29 (12%)</td>
<td>0.611</td>
</tr>
<tr>
<td>ER status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>76</td>
<td>10 (13%)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>174</td>
<td>19 (11%)</td>
<td></td>
</tr>
<tr>
<td>PR status</td>
<td></td>
<td></td>
<td>0.358</td>
</tr>
<tr>
<td>Negative</td>
<td>101</td>
<td>14 (14%)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>149</td>
<td>15 (10%)</td>
<td></td>
</tr>
<tr>
<td>HER2 status</td>
<td></td>
<td></td>
<td>0.873</td>
</tr>
<tr>
<td>Negative</td>
<td>143</td>
<td>18 (13%)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>76</td>
<td>9 (12%)</td>
<td></td>
</tr>
<tr>
<td>Metastatic site</td>
<td></td>
<td></td>
<td>0.482</td>
</tr>
<tr>
<td>Visceral</td>
<td>98</td>
<td>13 (13%)</td>
<td></td>
</tr>
<tr>
<td>Bone</td>
<td>35</td>
<td>2 (6%)</td>
<td></td>
</tr>
<tr>
<td>Both</td>
<td>118</td>
<td>14 (12%)</td>
<td></td>
</tr>
<tr>
<td>Extent of metastatic disease</td>
<td></td>
<td></td>
<td>0.768</td>
</tr>
<tr>
<td>One site</td>
<td>84</td>
<td>9 (11%)</td>
<td></td>
</tr>
<tr>
<td>Multiple sites</td>
<td>167</td>
<td>20 (12%)</td>
<td>0.249</td>
</tr>
<tr>
<td>Therapeutic setting</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
Elevated RAS p21 was significantly associated with shorter progression-free and overall survival in the univariate analysis (median PFS: 3.9 months [95%-CI: 1.8-6.0] for patients with elevated RAS p21 levels versus 8.5 months [95%-CI: 7.4-9.5] with non-elevated levels [p = 0.01]; median OS: 7.1 months [95%-CI: 0.3-14.2] versus not reached [p = 0.002], respectively). When RAS p21 cutoffs other than 452 pg/ml were considered, elevated RAS p21 was significantly associated with OS (p = 0.019 in case of 229 pg/ml; p = 0.003 in case of 320 pg/ml), but not with PFS. Classical clinical-pathological factors were included into a multivariate Cox regression analysis. In addition, factors previously shown to influence survival in a univariate analysis, such as serum HER2, CAIX and TIMP1, were included as well. In the multivariate analysis, RAS p21, presence of ≥ 5 CTCs per 7.5 ml blood, higher grading and higher line of therapy remained independent predictors of shorter OS.

**Conclusions**

This is the first study to address the clinical relevance of circulating RAS p21 in a large group of metastatic breast cancer patients. Patients with elevated levels of circulating RAS p21 had significantly worse clinical outcome. Hypothetically, these patients might benefit from therapeutic strategies targeting RAS pathway.
**Title:** Use of cell-free circulating RNA and expression of PD-L1 and HER2 in plasma to monitor and predict clinical response in metastatic breast cancer patients

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**Body:** Background: In addition to traditional radiology tests, cell-free circulating tumor RNA (cfRNA) extracted from plasma of cancer patients (pts) provides a means of evaluating tumor response, but based on molecular changes in the tumor. Measuring dynamic changes in gene expression and levels of total cfRNA (per ml of plasma) in metastatic patients has shown great potential for evaluating disease status and predicting outcome to anti-tumoral therapy in advance of imaging. Though checkpoint inhibitors have not been assessed widely in breast cancer, TNBC has shown mild responses to pembrolizumab and atezolizumab, with significantly better responses in pts with detectable PD-L1 expression.

**Methods:** Blood was drawn from pts at approximately 6-week intervals under various therapies and CT scans were performed at approximately 3-month intervals. CfRNA was extracted from the resulting plasma and reverse transcribed with random hexamers to cDNA. Levels of cfRNA were quantitated by RT-qPCR and correlated with pt response (PR/SD/PD), as determined by CT scans. Levels of gene expression in cfRNA (including PD-L1 and HER2) were monitored in pts across blood draws.

**Results:** A total of 28 breast cancer pts were enrolled in a 1-year clinical study. Of pts, 39% (11/28) were Caucasian and 36% (10/28) Hispanic. 19 pts completed the first two cycles of therapy: 2 pts had PR and showed no change (NC) or decrease (DEC) in levels of cfRNA, 11 pts achieved SD with 8 showing DEC or NC in cfRNA levels, and 6 pts had PD and all underwent increases (INC) in cfRNA levels (median increase: 788 ng/mL plasma) which correlated with progressive disease status. Of pts with SD/PR, 4 showed either an emergence or significant increase in PD-L1 expression across blood draws (3.7-98 ct); of PD pts, 1 showed a significant emergence of PD-L1 expression (12.5 ct) across blood draws. 3/5 of these PD-L1 expressing pts were being treated with an everolimus combination; the emergence or increase of PD-L1 in response to this therapy suggests use of checkpoint inhibitors as an option for these pts. In response to therapy, 3 of 5 pts had PD-L1 cfRNA levels above levels predictive of response to nivolumab in lung cancer pts. In the only pt with hyperexpressed HER2, the disappearance of HER2 cfRNA matched positive response (PR) to treatment with trastuzumab. PD-L1 decreased concomitantly for this pt.

**Conclusion:** We found a strong correlation between clinical responses and changes in plasma levels of ctRNA in breast cancer (84%). Most of these were documented several weeks before imaging was done. Levels of PD-L1 and HER2 expression in plasma can also be used to monitor pt response to specific therapies. The emergence of PD-L1 expression in response to various therapies in breast cancer may confer sensitivity to checkpoint inhibitor therapy.
2017 San Antonio Breast Cancer Symposium

Publication Number: P2-02-17

Title: Circulating tumor DNA analysis with ultra-high sensitivity sequencing in metastatic breast cancer

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Body: Introduction.
Circulating tumor DNA analysis has the potential to transform the clinical management of patients with breast cancer. We assessed the accuracy of ultra-high sensitivity ctDNA testing in patients with advanced breast cancer.

Methods.
From a prospective tissue collection study, we identified 25 patients with a contemporaneous metastatic tissue biopsy and plasma for ctDNA testing. Tumour DNA from the metastatic tissue biopsy was sequenced with a validated clinical hybrid capture panel, while plasma cell free DNA was sequenced with AVENIO ctDNA technology – a molecular barcoded duplex sequencing based on CAPPseq technology. Sample collection is on-going and results from the full concordance series will be presented at the conference.

Results.
Circulating tumour DNA was detectable in 87% (20/23) of patients, with at least one variant from tissue sequencing identified in plasma. There was overall high agreement between tissue and plasma sequencing. The sensitivity of plasma testing for variants identified in tumour, positive percent agreement, was 75% (24/32). Plasma testing revealed a diversity of sub-clonal mutations including polyclonal ESR1, polyclonal FGFR2 and FGFR3 mutations, rare KRAS mutations, and TSC1 and MSH2 inactivating mutations.

Conclusions.
Circulating tumour DNA testing with molecular barcoded duplex sequencing offers high sensitivity for tumour variant detection. The extent of sub-clonal resistance mutations identified emphasises the genetic diversity of advanced breast cancer.
Title: Higher mutation burden and mutant allele fraction of circulating tumor DNA corresponds to worse progression free survival in metastatic breast cancer patients

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Body: Introduction: Genomic profiling of circulating tumor DNA (ctDNA) allows non-invasive monitoring of tumor genetic changes and molecular heterogeneity. In addition to actionable mutations, mutational landscape derived from ctDNA could provide a better representation of overall tumor burden and tumor heterogeneity, as well as potentially impact clinical outcomes. To evaluate this hypothesis, this study assessed the association of mutation burden and average mutant allele fraction (MAF) with tumor subtype, therapeutic response, and survival in patients with metastatic breast cancer.

Methods: Whole blood samples from patients with metastatic breast cancer were collected during clinic visits before start of a new therapy. Plasma-derived cell-free DNA underwent complete next-generation sequencing of 73 cancer-related genes with the Guardant360 test. Mutation burden was defined as the number of genes with mutations, and average MAF was calculated as the sum of the highest MAF for each mutated gene divided by the number of genes with mutations. Time to progression was measured from the date of new treatment initiation after circulating tumor DNA collection to the date of progression. Multivariate cox proportional hazard models assessed the association of mutation burden and average MAF with progression free survival (PFS), adjusted for age, receptor subtype (hormone receptor positive, HR+; human epidermal growth factor 2 positive, HER2+; triple negative breast cancer, TNBC), treatment subtype (chemotherapy vs. targeted therapy), and number of prior metastatic breast cancer therapies. A p value of 0.05 was considered statistically significant.

Results: The study population consisted of 158 women with metastatic breast cancer (108 HR+, 14 HER2+, 19 TNBC) with a median age of 59 years and a median of 2 prior metastatic breast cancer therapies. Median follow up time was 4.0 months, and median PFS was 15.7 months. Mutation burden was greater in triple negative compared to hormone receptor positive breast cancer (7.5 vs. 4.8, p = 0.02) but no different in patients with > 2 prior metastatic therapies vs. not (5.1 vs. 4.7, p = 0.60) and age >45 vs. not (5.0 vs. 3.9; p = 0.26). In univariate models, high mutation burden (> median of 2) and high MAF (> median of 1.4) were significantly associated with worse PFS (Table). These results were similar in effect size and significance when adjusted for age, receptor subtype, treatment subtype, and number of prior metastatic breast cancer therapies. Impact of mutation burden on response to specific therapies will be presented at the meeting.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard Ratio</th>
<th>95% Confidence Interval</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>High mutation burden</td>
<td>1.99</td>
<td>1.12-3.54</td>
<td>0.02</td>
</tr>
<tr>
<td>High mutant allele fraction</td>
<td>1.88</td>
<td>1.06-3.33</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Conclusions: Higher ctDNA mutation burden and average MAF is associated with worse progression free survival and possibly reflects a more treatment refractory phenotype. Whether immunotherapy, alone or in combination, could influence the clinical outcomes in metastatic breast cancer patients with high ctDNA mutation burden is unclear and warrants additional research.
Title: Circulating tumor DNA detection anticipates disease recurrence in early stage breast cancer: A pilot study generating an observational confirmatory trial

Maria Grazia Daidone¹, Serena Di Cosimo¹, Silvia Veneroni¹, Filippo Cascone¹, Loris De Cecco¹, Matteo Dugo¹, Secondo Folli¹, Giulia V Bianchi¹, Elena Tamborini¹, Adele Busico¹ and Valentina Appierto¹. ¹Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy.

Body: Sensitive tumor biomarkers able to monitor disease progression would contribute to post-surgical treatment decision making in early breast cancer. We investigated the feasibility of using circulating tumor DNA (ctDNA) to early detect new disease manifestations in serial plasma samples collected during post-surgery follow-up from patients operated for stage I breast cancer from 1992 to 1993 at Istituto Nazionale Tumori in Milan. Forty patients that underwent radical or conservative surgery for T1/T2-N0-M0 breast cancer and that were followed for at least 15 years were included in a pilot study for the retrospective analysis of ctDNA on at least 3 plasma samples obtained during follow-up. To assess the feasibility of ctDNA analysis in archival plasma samples collected in heparin and stored from 10 to 25 years, preliminary experiments demonstrated that ctDNA was not affected by: 1) heparinase I digestion of extracted DNA and 2) DNA pre-amplification step to overcome limitations due to small plasma aliquots. Mutational analysis of breast cancer tissues was performed by Ion Torrent-targeted next generation sequencing and the identified Single Nucleotide Variations (SNV) were first validated and then tracked in plasma samples by using ad hoc digital polymerase chain reaction assays. One or more SNVs were identified in tumor tissue specimens and validated in 27/40 cases. Among those 27 breast cancers, 6 cases relapsed locally, 4 in distant sites, and 17 remained disease-free for the entire follow-up. ctDNA was undetectable during the post-surgical follow-up in 16/17 disease-free women up to 160 months of surgery, while it was detectable in 9/10 patients developing unfavorable events and anticipated the clinical diagnosis of relapse in 7/10 patients with a median lead time of 20 months. Our results are the first to associate mutation tracking to local recurrence and indicate that in patients with early breast cancer ctDNA monitoring during post-operative follow-up can anticipate the diagnosis of new disease manifestations, thus potentially allowing prompt treatments. These findings establish the rational to plan prospective studies to evaluate in the early breast cancer context the potential of ctDNA as a non-invasive and sensitive biomarker for monitoring tumor progression. Based on these results, we activated in 2016 a prospective observational study to confirm the predictive value of ctDNA on local and distant relapse in patients with early and localized triple negative breast cancer. As for May 2017, 145 patients with triple negative tumors were potentially enrolled for a ctDNA-based post-surgical follow-up: 111 cases at first diagnosis and 34 cases at surgery after neo-adjuvant treatment. One hundred-ten women accepted to participate in the study and signed a specific informed consent, whereas 22 patients refused to participate and 13 were lost to follow-up. For 94 patients (66 at initial diagnosis and 28 after neoadjuvant chemotherapy) plasma samples have been already longitudinally collected and DNA sequencing is currently in progress.
Title: The utility and correlation of circulating tumor cells (CTCs) and cell-free circulating tumor DNA (ctDNA) based on HER2 positivity

Body: Background:
CTCs are well-established prognostic and predictive biomarkers for metastatic breast cancer (MBC) and other solid tumors. ctDNA is emerging as a quantitative blood-based biomarker for monitoring genomic alterations and disease progression. We evaluated the clinical utility and correlation of these liquid biopsy molecular tools in a cohort of MBC patients.

Methods:
CTC samples were obtained from an ongoing, prospective study of blood based prognostic biomarkers for breast cancer patients. At this time, 71 patients and 98 total samples have been collected. CTC enumeration was performed using the CellSearch™ platform (Menarini, IT). Within this cohort, MBC patients who had ctDNA testing were identified. ctDNA testing was performed using Guardant360™ (Guardant Health, CA), a digital next-generation sequencing technology. Two groups were analyzed: (1) HER2-negative patients with CTC ≥ 5 in 7.5 ml of blood (2) HER2-positive patients who had been treated with HER2 targeted therapy.

Results:
22 samples (N=16 patients) were found with CTC ≥ 5 (range 8-904) and concurrent ctDNA testing (median timeframe between collection 0 days, range 0-42 days). There was a significant association between number of CTCs and the total number of genomic alterations detected in ctDNA (paired two sample t-test, p=0.012). In addition, CTC enumeration was significantly correlated with somatic alteration burden of the dominant clone (paired two sample t-test, p=0.023). The most common alterations detected in the blood were TP53 (55% of patients, 18 total mutations), PIK3CA (41% of patients, 15 total mutations), and ESR1 (32% of patients, 14 total mutations). For patients with HER2 positivity receiving HER2-targeted therapies (N=16 samples from 11 patients), only 18.8% of samples had detectable CTCs (all less than 5) as compared to 75.0% of samples with detectable ctDNA alterations. In N=12 samples with detectable ctDNA mutations, mean number of genomic alterations was 4.4 with mean somatic mutation burden of 2.95%.

<table>
<thead>
<tr>
<th></th>
<th>CTCs detected</th>
<th>ctDNA detected</th>
<th>CTC ≥ 5</th>
<th>Mean number of ctDNA alterations+</th>
<th>Mean somatic alteration burden+</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2- (only cases with CTC ≥ 5)</td>
<td>100% (22/22)</td>
<td>100% (22/22)</td>
<td>100%</td>
<td>6.7</td>
<td>16.1%</td>
</tr>
<tr>
<td>HER2+ (all cases)</td>
<td>18.8% (3/16)</td>
<td>75.0% (12/16)</td>
<td>0%</td>
<td>4.4</td>
<td>2.95%</td>
</tr>
</tbody>
</table>

+excludes ctDNA samples without detected genomic alterations

Conclusions:
In HER2-negative MBC patients, CTC enumeration was significantly correlated with the number of ctDNA genomic alterations and somatic alteration burden, indicating the potential for ctDNA as a prognostic, quantitative biomarker of tumor burden. In patients with HER2 positivity, ctDNA may be a more sensitive liquid biopsy tool given the rarity of detecting CTCs detection in this population using the CellSearch™ system. In HER2-positive patients, consideration of size-dependent selection of CTCs using filtration of cells that have undergone epithelial-mesenchymal transition may improve detection in this subgroup.
2017 San Antonio Breast Cancer Symposium

Publication Number: P2-03-01

Title: Analytical validation of a standardized scoring protocol for Ki67 assessed on breast excision whole sections: An international multicenter collaboration

Torsten O Nielsen1, Samuel CY Leung1, Lila A Zabaglo2, Indu Arun3, Sunil S Badve4, Anita L Bane5, John MS Bartlett6, Signe Borqquist7, Martin C Chang8, Andrew Dodson9, Anna Ehinger2, Susan Fineberg10, Cornelia M Focke11, Dongxia Gao1, Allen M Gown12, Carolina Gutierrez12, Judith C Hugh14, Zuzana Kos15, Anne-Vibeke Lænholm16, Mauro G Mastropasqua17, Takuya Moriya18, Sharon Nofech-Mozes19, C Kent Osborne23, Frédérique M Penault-Llorca20, Tammy Piper21, Takashi Sakatani22, Roberto Salgado23, Jane Starczynski24, Tomoharu Sugie25, Bert van der Vegt26, Giuseppe Viale17,27, Daniel F Hayes28, Lisa M McShane29 and Mitch Dowsett2. 1University of British Columbia, Vancouver, BC, Canada; 2The Institute of Cancer Research, London, United Kingdom; 3Tata Medical Center, Kolkata, West Bengal, India; 4Indiana University Simon Cancer Center, Indianapolis, IN; 5Juravinski Hospital and Cancer Centre, McMaster University, Hamilton, ON, Canada; 6Ontario Institute for Cancer Research, Toronto, ON, Canada; 7Lund University, Lund, Sweden; 8Sinai Health System and University of Toronto, Toronto, ON, Canada; 9Ralph Lauren Centre for Breast Cancer Research, The Royal Marsden Hospital, London, United Kingdom; 10Montefiore Medical Center and the Albert Einstein College of Medicine, Bronx, NY; 11Dietrich-Bonhoeffer Medical Center, Neubrandenburg, Mecklenburg-Vorpommern, Germany; 12PhenoPath Laboratories, Seattle, WA; 13Lester and Sue Smith Breast Center and Dan L. Duncan Comprehensive Cancer Center, Baylor College of Medicine, Houston, TX; 14University of Alberta, Edmonton, AB, Canada; 15University of Ottawa and The Ottawa Hospital, Ottawa, ON, Canada; 16Zealand University Hospital, Slagelse, Region Sjælland, Denmark; 17European Institute of Oncology, Milan, Italy; 18Kawasaki Medical School, Kurashiki, Okayama Prefecture, Japan; 19University of Toronto Sunnybrook Health Sciences Centre, Toronto, ON, Canada; 20Centre Jean Perrin and Université d’Auvergne, Clermont-Ferrand, France; 21Edinburgh Cancer Research Centre, Western General Hospital, Edinburgh, United Kingdom; 22Nippon Medical School, Bunkyo-ku, Tokyo, Japan; 23Institut Jules Bordet, Brussels, Belgium; 24Birmingham Heart of England, National Health Service, Birmingham, United Kingdom; 25Kansai Medical University, Hirakata, Osaka, Japan; 26University Medical Center Groningen, Groningen, Netherlands; 27University of Milan, Milan, Italy; 28University of Michigan Comprehensive Cancer Center, Ann Arbor, MI and 29National Cancer Institute, Bethesda, MD.

Body: Aims: (i) Determine whether between-observer reproducibility for Ki67 when assessed on whole sections according to a standardized scoring protocol is adequate for clinical application. (ii) Compare between-observer reproducibility of Ki67 scores assessed on hot-spots to scores using a global method that averages across a tissue section.

Background: The nuclear proliferation biomarker Ki67 has multiple potential roles in breast cancer, including aiding decisions based on prognosis, but unacceptable levels of between-laboratory variability have been observed. The International Ki67 in Breast Cancer Working Group has undertaken a systematic program to determine whether Ki67 measurement can be analytically validated and standardized across labs. In phase 1, variability in visual interpretation was identified as an important source of variability. Phases 2 and 3a showed that adherence to defined scoring methods substantially improved reproducibility in scoring tissue microarrays and core-cut biopsies. We now assess whether acceptable reproducibility can be achieved on whole sections.

Methods: Adjacent sections from 30 primary ER+ breast cancers were centrally stained for Ki67 to assemble 4 sets of 30 stained tumor sections, circulated among 23 labs in 12 countries. Ki67 was scored by 2 methods by all labs: (a) global: 4 fields of 100 tumor cells each were selected to reflect observed heterogeneity in nuclear staining (b) hot-spot: the field with highest Ki67 percentage of tumor cells with nuclear staining was selected and up to 500 cells scored. Ki67 scores were log2-transformed for statistical analyses and back-transformed for presentation. The primary objective was to assess whether either method could achieve an intraclass correlation coefficient (ICC) significantly greater than 0.8, considered substantial to almost-perfect reproducibility. Secondary objectives were to assess which method had highest observed ICC and to assess whether observers identified the same “hot-spots”.

Results: ICC for the global method was 0.87 (95%CI: 0.799-0.93), marginally meeting the prespecified success criterion. The ICC for the hot-spot method was 0.83 (95%CI: 0.74-0.90) and had a CI extending below the success criterion. Across the 23 labs, geometric mean value of the 30 scores ranged from 8.5 to 19.6 for the global method and from 12.8 to 30.3 for the hot-spot method. The overall mean (95% CI) of these values was 12.9 (11.9-14.0) and 20.9 (19.1-22.8), respectively. Visually, between-laboratory agreement in location of selected hot-spot varies between cases. The median times for scoring were 9 and 6 minutes for global and hot-spot methods respectively.
Conclusions: The global method marginally met the prespecified criterion of success; it should now be evaluated for clinical validity in appropriate cohorts of cases. The hot-spot method was observed to have slightly less reproducibility between labs. The time taken for scoring by either method is practical using counting software we are making publicly available. Establishment of external quality assessment schemes is likely to improve the reproducibility between labs further.

(Supported by a grant from the Breast Cancer Research Foundation)
Title: Macrodissection prior to closed system RT-qPCR is not necessary for estrogen receptor and HER2 concordance with IHC/FISH in breast cancer

Swati Gupta¹, Daniel E Carvajal-Hausdorf¹, Brad E Wasserman¹, Kenneth Ho², Jodi Weidler², Wendy Wong², Brian Rhees², Michael Bates² and David L Rimm¹. ¹Yale University School of Medicine, New Haven, CT and ²Division of Oncology Research and Development, and Medical and Scientific Affairs and Strategy, Oncology, Cepheid, Sunnyvale, CA.

Body: Background: An on-demand, closed system RT-qPCR (the GeneXpert system, Cepheid, Sunnyvale, CA) has the potential to provide biomarker information in low resourced settings. The system consists of an inexpensive, single-use, disposable, macrofluidic cartridge and an instrument that automates RT-qPCR. Here we use it with a research use only cartridge (STRAT4) that measures the mRNA expression levels of ESR1, PGR, ERBB2, and MKi67 using a single 5uM thick FFPE tissue section from an excisional or core biopsy specimen containing invasive carcinoma of the breast. The assay, results are expressed as a delta cycle threshold (dCt) value, defined as the Ct of a control gene (CYFIP1) minus the Ct of the target gene (ESR1, PGR, ERBB2, or MKi67). We determine whether the dCt result for each marker is equivalent using the entire non-macrodissected section (non m-d) to the dCt results obtained following tumor macro-dissection (m-d) to eliminate non-tumor elements from the assay.

Methods: We evaluated the impact of m-d versus non m-d using STRAT4 on a cohort of 62 formalin-fixed paraffin-embedded (FFPE) tumor core needle biopsy specimens with a range of HER2 expression determined by clinical immunohistochemistry and fluorescence in situ hybridization (IHC/FISH). Concordance (sensitivity and specificity) of the STRAT4 ESR1 and HER2 mRNA versus ER and HER2 IHC/FISH measurements were also assessed.

Results: We observed excellent agreement of the resulting dCt between the paired samples, m-d versus non m-d, for ESR1 (R²=0.92), PGR (R²=0.90), ERBB2 (R²=0.94) and MKi67 (R²=0.90). No significant difference (P value > 0.99) was observed when we compared the dCt between the paired samples m-d versus non m-d. In addition, using the predefined STRAT4 dCt cutoff for ESR and ERBB2 positivity, we found a significant concordance between RT-qPCR and IHC/FISH for ESR-positivity for the paired samples, m-d (P value < 0.001; sensitivity = 0.98; specificity = 1; PPV = 1; NPV = 0.95) versus non m-d (P value < 0.001; sensitivity = 0.98; specificity = 1; PPV = 1; NPV = 0.95) and HER2-positivity for the paired samples, m-d (P value < 0.001; sensitivity = 0.85; specificity = 0.98; PPV = 0.92; NPV = 0.96) versus non m-d (P value < 0.001; sensitivity = 0.71; specificity = 0.98; PPV = 0.90; NPV = 0.92), respectively.

Conclusion: These data suggest that mRNA for ESR and ERBB2 is sufficiently low in surrounding tissues that m-d of whole sections is not required for accurate assessment of key breast cancer mRNA markers in a closed system RT-qPCR assay. The simplicity of the assay workflow may be particularly valuable in low resourced settings where routine access to pathology expertise and to high quality IHC/FISH is challenging.
Title: A multicenter clinical study of Xpert® breast cancer STRAT4 demonstrates high concordance with central lab ER, PgR, HER2, and Ki67 IHC and HER2 FISH tests in FFPE breast tumor tissues

Natalie C Wu¹, Edith Wong¹, Barbara Acca¹, Jill Birkmeier¹, Lisa Tran¹, Suling Zhao¹, Wendy Wong¹, Victor C Chu¹, Ken Ho¹, Monisha Malek¹, Cici Lu¹, Grace Ge¹, Kerstin David³, Neil B Quigley⁴, Safedin Sajo Beqaj⁵, Simon Davenport², Jodi Weidler¹, Michael Bates¹ and Michael Press². ¹Cepheid, Sunnyvale, CA; ²University of Southern California, Los Angeles, CA; ³Indivumed GmbH, Hamburg, Germany; ⁴Molecular Pathology Laboratory Network, Maryville, TN and ⁵Molecular Testing Lab, Vancouver, WA.

Body: The Xpert® Breast Cancer STRAT4 (STRAT4) is a CE-IVD marked, semi-quantitative, cartridge-based RT-qPCR assay for the detection of ESR1, PGR, ERBB2 (HER2), and MKi67 mRNAs from formalin fixed, paraffin embedded (FFPE) breast tumors. The assay is fast (< 2 hrs), reproducible, robust, and easy to perform. The aim of this multicenter clinical study was to assess the performance characteristics of the STRAT4 assay relative to central lab immunohistochemistry (IHC) for ER, PgR, HER2, and Ki67 and to fluorescence in situ hybridization (FISH) for HER2 gene amplification.

Methods: A total of 200 archived primary invasive breast cancer FFPE blocks were sourced from Indivumed for this study. From each block, twelve (12) adjacent tissue sections (4-µm thickness) on slides were prepared for pathological H&E confirmation to define tumor area, and for testing by STRAT4, IHC (ER, PgR, HER2, Ki67), and HER2 FISH. Standard STRAT4 lysate preparation using a single unstained slide per specimen and testing on N=84, N=68, and N=48 samples was performed at 3 independent sites, respectively (2 US and 1 EU). A single slide from each specimen was also processed using the recommended concentrated lysate procedure for STRAT4 testing at Cepheid. All IHC and FISH testing was performed by a central academic reference laboratory in the US. For a given sample, STRAT4 data generated using the standard lysate procedure was included for concordance analysis when all target gene test results were valid. In cases where the standard lysate preparation yielded indeterminate test results for any target, data from the concentrated lysate preparation was used for the data analysis. Receiver Operating Characteristic (ROC) analysis, overall percent agreement (OPA), positive percent agreement (PPA), and negative percent agreement (NPA) between STRAT4 and IHC (IHC/FISH for HER2) were determined for ESR1, PGR, ERBB2, and MKi67.

Results: Of the 200 samples tested by STRAT4, all samples generated valid results for ESR1 and ERBB2, 199 of 200 samples were valid for PGR, and 198 of 200 samples were valid for MKi67 using the standard or concentrated lysate preparation protocol. One sample failed to generate results for both ER and PgR IHC. Twelve samples failed to yield HER2 FISH results. The STRAT4 success rate and results concordance with IHC were comparable across study sites. OPA between STRAT4 and IHC was 97% for ESR1, 88.9% for PGR, 93.3% for HER2 (92.4% for IHC and FISH), and 90.7% for MKi67 (excluding 10-20% staining). Areas under the ROC curves were 0.9922 for ESR1, 0.9509 for PGR, 0.9958 for ERBB2, and 0.9395 for MKi67.

Conclusion: STRAT4 measurements for ESR1, PGR, ERBB2 and MKi67 mRNA expression are robust and highly concordant with IHC (IHC/FISH for HER2). The technical portion of the assay is easily performed in < 2 hrs including hands-on time using standard FFPE tissue sections. Xpert STRAT4 offers local pathology labs an alternative to centralized, subjective IHC/FISH tests that require a higher level of expertise. Further investigations correlating STRAT4 markers directly with clinical outcomes in independent cohorts are in progress.
Title: Lymphovascular invasion in breast carcinoma following neoadjuvant chemotherapy is a strong prognosis factor

Anne-Sophie Hamy-Petit, Giang-Thanh Lam, Enora Laas, Lauren Darrigues, Thomas Balezeau, Julien Guerin, Alain Livartowski, Benjamin Sadacca, Jean-Yves Pierga, Anne Vincent-Salomon, François-Clement Bidard, Florence Lerebours, Etienne Brain, Veronique Becette, Roman Rouzier, Marick Lae and Fabien Reyal. 1Institut Curie, Paris, France and 2Geneva University Hospitals, Geneva, Switzerland.

Body: Purpose: Lymphovascular invasion (LVI) is a poor prognosis factor in breast cancer (BC), but data on its value in the neoadjuvant setting is scarce. This study evaluates the relationships between post-NAC LVI and prognosis in BC.

Methods: We identified 1197 patients with primary BC receiving NAC +/- trastuzumab between 2002 and 2011. Information on LVI in post-NAC surgical specimen was retrieved from review of medical charts. Univariate and multivariate analyses were performed to assess the association of clinical, pathological factors with disease free survival (DFS) and overall survival (OS) was assessed using a cox proportional hazard model.

Results: On 1197 tumors, 528 were luminal (44.1%), 375 were triple negative breast cancer (TNBC) (31.3%) and 294 were HER2-positive (24.6%). On post-NAC surgical specimens, LVI was present in 302 (25.2%), absent in 531 (44.4%), and was not mentioned in 364 cases (30.4%). The presence of post-NAC LVI was associated with an impaired DFS (HR=2.17, 95 CI [1.65 - 2.86], p<0.001) and the magnitude of this impact varied by BC subtype (p-value for interaction=0.02), (luminal BC: HR=1.75, p=0.006; TNBC : HR=2.77, p<0.001 ; HER2-positive BC : HR=5.12, p<0.001).

Table 1 Univariate analysis and multivariate analysis on DFS (whole population)

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<td>3 5.21</td>
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**Post NAC parameters**

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<td>2 1.69</td>
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<td>3 5.21</td>
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</table>

ER: oestrogen receptor PR: progesteron receptor RCB: residual cancer burden

Post-NAC LVI was an independent predictor of poor DFS, that overwhelmed the prognostic impact of pathological complete response in all 3 BC subtypes. Post-NAC LVI was also an independent predictor of poor OS in the whole cohort and in all BC subtypes.

Table 1 resumes univariate and multivariate analysis on DFS in whole population.

**Conclusion:** Post-NAC LVI is a strong independent prognostic factor associated with poor DFS and OS, that (i) should be systematically mentioned in pathological reports following NAC and (ii) could be used to select high risk patients candidates to second line trials in the post-neoadjuvant window.
**Title:** AJCC prognostic stage including oncotype DX®RS: Impact on breast cancer staging compared with traditional anatomic TNM stage

Roisin M O’Cearbhaill¹, John M Gannon¹, Liam A Devane¹, Ruth S Prichard¹, Janice Walshe¹, Enda W McDermott¹ and Cecily M Quinn¹. ¹St Vincent's University Hospital, Dublin, Ireland.

**Body:**

**INTRODUCTION**

The recently published AJCC Cancer Staging Manual 8th edition, scheduled to be adopted into clinical practice in January 2018, proposes a new breast cancer (BC) Prognostic stage that includes histological grade, estrogen receptor (ER), progesterone receptor (PR), and HER2 status in addition to the traditional Anatomic TNM stage. In a further modification, patients with ER positive, HER2 negative, lymph node negative BC and an Oncotype DX® recurrence score (RS) of less than 11 will be assigned to Prognostic stage 1A irrespective of histological grade and size (up to 5cm). The changes have been proposed in recognition of the prognostic influence of these parameters and in attempt to improve personalised patient care. The aim of this study is to profile the impact on staging by comparing Prognostic stage, with and without RS, to traditional Anatomic stage, in a series of patients with early BC and an RS of less than 11.

**METHODS**

The study population comprised 127 patients with primary BC and an RS of less than 11, derived from a consecutive series of 729 patients with ER positive, Her2 negative, lymph node negative, primary BC whose tumors were tested using the Oncotype DX® multigene assay between November 2011 and December 2016. The median patient age was 57 years (35-78). Pathological tumour size was T1a (n=2), T1b (n=19), T1c (n=79) and T2 (n= 27). Median tumor size was 15mm. Histological tumour grade was grade 1 (n=14), 2 (n=98) and 3 (n=15).

Each patient was assigned an Anatomic and Prognostic stage, with and without RS modification, according to the AJCC Classification of Cancer Staging, 8th edition. The three staging categories were compared and the impact on stage profiled.

**RESULTS**

Applying the traditional Anatomic Stage (TNM), 100 patients were assigned to stage IA (T1N0) and 27 patients to stage IIA (T2N0). Applying the Prognostic stage (without RS) 89 patients were assigned to stage IA, 33 to stage IB, four to stage IIA, and one to stage IIB. All patients were assigned to stage IA according to the Prognostic stage that includes a RS of < 11.

Comparing Prognostic stage without RS to traditional Anatomic stage, 26.7% of patients (n=34) underwent a change in stage, 9.4% (n=12) to a higher stage and 17.3% (n=22) to a lower stage. 21.3% (n=27) of patients were down staged when comparing Prognostic stage including RS to traditional Anatomic stage. Comparing Prognostic stage including RS to Prognostic stage without RS, 29.9% (n=38) of patients were down staged.

**CONCLUSION**

Breast cancer staging is an important tool in guiding patient management. This study demonstrates that the introduction of the Prognostic stage and the modified Prognostic stage to include RS will alter the assigned stage in approximately 25% of patients with early BC.

**REFERENCE**

Title: Draft recommendations for human epidermal growth factor receptor 2 (HER2) testing in breast cancer will decrease HER2 positivity rates

Eric Johnson1 and Evin Gulbahce1. 1University of Utah, Salt Lake City, UT.

Body: Introduction: A focused update of American Society of Clinical Oncology (ASCO) and College of American Pathologist’s (CAP) guideline recommendations for HER2 testing in breast cancer (BrCa) was published in May, 2017 for public comment (http://www.cap.org/ShowProperty?nodePath=/UCMCon/Contribution Folders/WebContent/pdf/her2-breast-summary-draft-recommendations-2017.pdf). The focused update for in situ hybridization (ISH) addresses following uncommon scenarios: 1) HER2/CEP17 ratio ≥ 2.0, and HER2 /cell <4.0; 2) HER2/CEP17 ratio <2.0 and HER2 /cell ≥6.0; 3) HER2/CEP17 <2.0 and HER2 /cell ≥4.0 and <6.0. The first two groups are currently reported as positive, the third as equivocal. It is recommended that immunohistochemistry (IHC) performed by the same lab for all these groups. If the IHC testing is 3+ or 0/1+ the final diagnosis will be reported as positive or negative respectively. If the reflex IHC is 2+, a recount of the original ISH area of carcinoma with IHC 2+ staining is recommended. The purpose of this study is to review the results of targeted FISH testing following IHC to predict the effects of proposed guidelines in a high volume national reference lab.

Materials and methods: HER2 FISH tests performed on BrCa between 4/2015-5/2017 are included. Our lab offers HER2 testing by IHC (HercepTest™ Dako) and dual probe FISH (Dako IQ). Equivocal (2+) cases showing ≥10% weak or moderate circumferential membrane staining or intense but <10% circumferential staining are circled by a pathologist and reflexed to HER2 FISH with preferential counting performed in the circled areas. Equivocal FISH cases are re-tested with the alternate RAI1 probe (Agilent Technologies). FISH scoring is done manually by 2 people following 2013 ASCO/CAP guidelines with at least 20 cells counted in amplified and non-amplified cases and 40 cells counted in equivocal cases.

Results: 2460 HER2 FISH test requests were received during the study period. 7 cases failed at initial testing and 13 cases failed at repeat testing of equivocal cases with reflex probe. 389 (16.2%) cases were amplified, 1686 (68.7%) non amplified, and 369 (15.0%) were equivocal with FDA approved probe set. 116 (32.6%) of equivocal cases re-tested with alternate probe were amplified increasing overall amplification rate to 21%. The table below shows cases that fall under 3 uncommon categories that are proposed for changes in draft guidelines.

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<th>Average HER2/Cell</th>
<th>Number of Cases with IHC (Total Number)</th>
<th>IHC n(result)</th>
<th>Results per 2013 ASCO/CAP before Reflex Probe</th>
<th>Results per 2013 ASCO/CAP after Reflex Probe</th>
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<td>1 (1+)</td>
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<td>&lt; 2 ≥ 6</td>
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<td>9 (31)</td>
<td>9 (2+)</td>
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<td>98 (372)</td>
<td>96 (2+)</td>
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Conclusions: In our lab where all FISH tests with prior IHC are counted on targeted areas, all of the cases within the first two groups will be reclassified as negative. The use of reflex probe in equivocal cases is not recommended in the draft recommendations further decreasing positivity rates. Approximately 5% decrease is expected in HER2 FISH positivity rates.
following proposed recommendations in reference laboratory setting.
Body: INTRODUCTION

Proliferative activity is one of the most important prognostic parameters in cancer. During the pathological examination of breast tumors, it is routinely evaluated by a count of the number of mitoses. Adding immunohistochemical stains of the nuclear protein Ki67 provides extra prognostic and predictive information. However, the currently used methods for both of these evaluations battle imperfections, primarily in reproducibility. In this study, we make an equally broad and detailed evaluation of mitoses, Ki67 and the more recently described Phosphohistone H3 (PHH3) in primary breast cancer using digital image analysis (DIA). Furthermore, we aim to investigate the prognostic and predictive value of proliferation-associated biomarkers in breast cancer stromal cells in relation to patient outcome.

MATERIALS AND METHODS

Two cohorts of primary breast cancer specimens (total n=297) with clinicopathological data including >10 years survival data, were sectioned and stained for Ki67, PHH3 and pancytokeratin (CKMNF116) and all glass slides were digitally scanned at x20. The DIA software used was the Visiopharm Integrator System (VIS) by Visiopharm A/S, Hoersholm, Denmark. VIS operates by a 'digital fusion' method that automatically excludes non-epithelial tissue restricting the analysis of the biomarkers (Ki67 and PHH3) to CKMNF116 positive cells. Both manual and DIA scores were compared for sensitivity and specificity for the gene expression based Luminal B versus A subtype, for high versus low transcriptomic grade as well as for their prognostic value in terms of Cox regression hazard ratios and breast cancer specific and overall survival. Further, we investigated whether the expression of Ki67 in the tumors’ hot spots, invasive edges or as an average across all regions should be assessed for maximum power in relation to these outcomes. In addition, by inverting the DIA algorithm run by the VIS on the same cohorts, the expression of Ki67 and PHH3 was evaluated in the tumor stromal compartment.

RESULTS

Regardless of tumor region, DIA of Ki67 outperformed the other markers in sensitivity and specificity for gene expression subtypes and transcriptomic grade. In contrast to mitotic counts, tumors with high expression of Ki67 as defined by DIA, had significantly increased hazard ratio for all-cause mortality within 10 years from diagnosis. DIA of Ki67 was superior to manual Ki67 and PHH3 evaluations as well as to mitotic counts in terms of separation of patients with poor versus relatively good survival. Finally, we replaced the manual mitotic counts with DIA of Ki67 in hot spots as the marker for proliferation when determining histological grade. This increased the differences in estimated mean overall survival between the highest and lowest grades and added significantly more prognostic information to the classic Nottingham histological grade.

CONCLUSIONS

We conclude that digital image analysis of Ki67 in hot spots should be suggested as the marker of choice for proliferative activity in breast cancer.
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Body: Background
HER2 targeted therapies have substantially improved survival outcomes for patients with breast cancer and HER2 amplification of their tumour. The recently updated American Society of Clinical Oncology/College of American Pathologists (ASCO CAP) guidelines recommend that HER2 testing to be performed on all invasive breast cancers and that positive HER2 status is defined by either evidence of HER2 protein over-expression measured by immunohistochemistry status (IHC 3+) or by in-situ hybridisation (ISH) amplification. Equivocal results require further testing with the alternative assay.
In Australia, Medicare-funded access to HER2 targeted therapies requires demonstration of HER2 positivity by ISH, regardless of IHC status. Thus, currently most breast units perform IHC and ISH on all breast cancers. Given this discrepancy in practice and significant additional cost of performing ISH, this study evaluated the frequency of discordance and the cost burden in using both assays in a historical series of patients with breast cancer.

Methods
A retrospective audit of HER2 histopathology reports was performed for all breast cancers diagnosed between 2007 and 2016 at a single tertiary hospital in Melbourne, Australia (The Austin Hospital). HER2 IHC results were classified as negative (0 or 1+), equivocal (2+) or positive (3+). An analysis was performed to assess the proportion of concordant (defined as samples with IHC 0/1+ and negative ISH, samples with IHC3+ and positive ISH, and samples with IHC2+ regardless of ISH) and discordant results (defined as IHC 3+ and ISH non-amplified or IHC 0/1+ and ISH amplified). Tumour blocks and slides from discordant cases were reviewed by a breast cancer histopathologist. The cost of additional testing in concordant cases was performed based on the Australian Medicare Benefits Schedule (MBS).

Results
Eight hundred and forty-six histopathology reports were reviewed from 786 patients, all of whom had both IHC and ISH assays. There were 832 (98.8%) concordant cases. There were 10 discordant cases (1.2%) in total, including three cases (0.4%) with a negative IHC (1+) result but positive ISH, and 7 cases (0.8%) with a positive IHC but negative ISH result. A detailed analysis of 10 discordant cases will be presented. HER2 status and subsequent treatment remain unchanged with the addition of ISH testing in 665 (79.0%) cases, which amounted to an additional $209,741 (AUD) being spent on unnecessary ISH testing. Given an estimated incidence of 17,730 new cases of breast cancer per year in Australia, the population cost of performing additional HER2 ISH testing ($315.40 per case) in concordant cases would equate to $4.4 million (AUD) per year.

Conclusion
These results describe an extremely low rate of HER2 IHC/ISH discordance suggesting that routine use of both assays is unnecessary in cases with an unequivocal IHC result. The fiscal burden and potential delays to deciding treatment provide a strong rationale for access to HER2 targeted therapies to be based IHC or ISH, as advised by the ASCO CAP guidelines. Our results will be used to support a national review of IHC and ISH discordance using Australian national registry data.
Title: An institutional look back: Maximizing the utility of MammaPrint® in invasive breast cancer diagnostics without breaking the bank

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Body: Subtyping of invasive breast cancer utilizing immunohistochemical stains for estrogen receptor, progesterone receptor, and HER2, with or without the proliferation marker Ki-67, is the current standard of care employed by pathologists. The development of the BluePrint® molecular subtyping assay by Agendia, a microarray-based gene expression test used in conjunction with MammaPrint, to molecularly subtype and assess recurrence risk in early-stage breast cancer, has breast surgeons and oncologists challenging the status quo.

Study Objectives: With increasing emphasis placed of proper use of declining resources, the current study aimed to determine parameters in which such testing has the greatest added value.

Materials and Methods: All invasive breast cancer biopsies and resection specimens with formalin fixed paraffin embedded tissue sent for testing at Agendia Genomics Laboratory from April 2015 to May 2017 were reviewed in an institutional look back. 87 cases were reviewed and conventional immunohistochemical cancer profiling, tumor size, and grade were compared with MammaPrint prognostic reports.

Results and Discussion: Of the 87 cases compared, 67 (77%) showed concordance between immunohistochemical and molecular subtyping. Of the 20 discordant cases, 13 (65%) were subtyped as low risk luminal A by Mammaprint/BluePrint despite demonstrating high risk clinicopathologic features including larger size (average 2 cm vs overall average 1.4 cm), higher grade (1 low grade, 11 intermediate grade, and 1 high grade vs totals of 36 low grade, 42 intermediate grade, and 20 high grade), and increased proliferation indices. 3 (15%) of discordant cases revealed HER2 amplification by fluorescence in situ hybridization. Near complete agreement was found among basal subtype and Luminal A tumors with Ki-67 less than 12%, indicating the lack of added value among these cases.

Conclusion: Study results validate the limited role of MammaPrint/BluePrint in cases of invasive breast cancer. Specifically, the greatest value of MammaPrint/BluePrint was recognized in luminal subtypes with intermediate grade and proliferation indices ranging from 12 – 29%.
Title: Risk factors for upgrading and upstaging of pre-operative biopsies in ductal carcinoma in situ

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Body: Background: Ductal carcinoma in situ (DCIS), accounts for one fifth of all screen-detected neoplastic breast lesions. Contemporary research in DCIS focuses on separating lesions that need active treatment from those that can be safely left under surveillance. This, in turn, relies on accurate determination of invasive status and DCIS grade at time of initial biopsy. Most previous studies have examined factors associated with upstaging the diagnosis from DCIS to invasive breast cancer (IBC) following surgery, and few have evaluated factors associated with upgrading the diagnosis to a higher grade of DCIS. This is because upgrading has not traditionally influenced clinical management in the way that upstaging has done. However, recent interest in non-operative treatment for low-risk DCIS has meant that accurate determination of grade at time of initial biopsy has become more important. We aimed to compare risk factors for upgrading and upstaging of biopsies in DCIS.

Method: We undertook a cohort study of all women diagnosed with DCIS at a large specialist cancer centre between 2000–2014. Information from the clinical records was abstracted, including the pre-operative mammography (MMG) and pathology information from the initial biopsy. We also abstracted pathology information regarding the excised specimen in order to identify women whose diagnosis was subsequently upgraded or upstaged. We looked for factors that were predictive for upgrading or upstaging.

Result: A total of 641 women were diagnosed with DCIS at initial biopsy. Of these, 72 (11%) were upgraded: 26 (4%) from grade 1 to grade 2, 2 (0.3%) from grade 1 to grade 3 and 44 (7%) from grade 2 to grade 3. A further 115 (18%) were upstaged to IBC: 20 of these (3%) had grade 1 DCIS on initial biopsy, 47 (7%) had grade 2, 43 (7%) grade 3, and for 5 (1%) biopsy grade was not available. Necrosis on biopsy increased the risk of upgrading (with necrosis: 14% upgraded, without: 10% upgraded, p for difference 0.02) and also of upstaging (with necrosis: 23% upstaged, without: 15% upstaged, p for difference <0.01). Lesions measuring ≥50 mm on MMG were more likely to be upgraded than smaller lesions (0-19 mm: 9% upgraded, 20-50 mm: 9% upgraded, ≥50 mm: 19% upgraded, p for heterogeneity <0.01), while lesions measuring 20-50 mm and ≥50 mm were both more likely to be upstaged than lesions measuring 0-19 mm (0-19 mm: 9% upstaged, 20-50 mm: 23% upstaged and ≥50 mm: 21% upstaged, p for heterogeneity <0.01). Fewer 9G vacuum-assisted biopsies than 14G core biopsies were upgraded (9G vacuum-assisted: 7% upgraded, 14G core: 15% upgraded, p for difference 0.01), while the effect of biopsy method on upstaging was not significant (9G vacuum-assisted: 12% upstaged, 14G core: 16% upstaged, p for difference 0.15). Presence of a palpable lump was not significantly associated with upgrading (palpable lump: 13% upgraded, no palpable lump: 10% upgraded, p for difference 0.19) but increased the risk of upstaging (palpable lump: 23% upstaged, no palpable lump: 16% upstaged, p for difference 0.02).

Conclusion: Our findings suggest that consideration of MMG lesion size and necrosis on biopsy may be helpful in selecting low-risk women for non-operative management of DCIS, as may use of the 9G vacuum-assisted method of biopsy.
The immune microenvironment of HER2-positive ductal carcinoma in situ of the breast

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Body: Background: Ductal carcinoma in situ (DCIS) consists of a heterogeneous group of tumors that can be subclassified based on their molecular profile similar to invasive breast carcinomas. DCIS ducts are often surrounded by an inflammatory infiltrate, yet few studies have focused on this inflammatory response in the different subtypes of DCIS. The role of the immune microenvironment in tumor progression and response to therapy has led to a number of effective immune-based therapies, particularly targeting the PD-1/PD-L1 axis, and these approaches may extend to breast cancer subtypes and their precursor DCIS. In this study we evaluated the inflammatory response and PD-1/PD-L1 expression in both tumor-infiltrating lymphocytes (TILs) and tumor cells in HER2-positive (HER2+) and HER2-negative (HER2-) DCIS.

Design: Our population consisted of 85 cases of pure DCIS treated with surgical excision or mastectomy at our institution from 2008 to 2012. The molecular subtypes of DCIS were determined based on ER and HER2 expression. Tissue microarrays (TMAs) were constructed with 3 cores from each case to account for tumor heterogeneity. The presence of tumor-associated inflammation (TAI) was graded as absent, mild (<10%), moderate (10-50%) or severe (>50%) and the extent and intensity of PD1 and PD-L1 in the TILs and in the tumor cells were also assessed. A composite score was calculated by multiplying extent and intensity (range 0-12 with 9-12=strong).

Results: Of the 85 cases, 51 were classified as Luminal A (ER+/HER2-), 15 as Luminal B (ER+/HER2+), 13 as HER2+ (ER-/HER2+) and 6 as basal-like (ER-/HER2-). Moderate/severe inflammation around DCIS correlated with HER2 expression (20/28 HER2-expressing cases (71.4 %) compared to 21/36 HER2-non-expressing cases (58.3%), p=0.005). Of interest, severe inflammation was seen almost exclusively around HER2-expressing cases (7/28, 25% vs 1/57, 1.7 %, p=0.002). Furthermore, over half of the TILs around HER2+ cases expressed PD-L1 (7/13, 54%), while only 6% of Luminal A (3/47) and none of Luminal B (0/15) or basal-like (0/6) cases did (p=0.00001). In addition, about 1/3 of the TILs around HER2+ cases expressed PD-1 (4/13, 31%), while only 8% of Luminal A (4/49) and none of Luminal B (0/15) or basal-like (0/6) cases did (p=0.004). None of the DCIS tumor cells expressed PD-1. Of interest, 15% of HER2+ cases (2/13) and 7% of Luminal B cases (1/15) expressed PD-L1 in the DCIS tumor cells.

Conclusions: 1. Moderate/severe inflammation around DCIS foci correlates with HER2 expression. 2. PD-L1 expression in TILs is seen almost exclusively in HER2+ DCIS cases. 3. About 1/3 of the TILs around HER2+ DCIS cases also express PD-1. 4. None of the DCIS tumor cells express PD-1. 5. Small subgroups of HER2+ and Luminal B DCIS cases show PD-L1 expression in the tumor cells themselves. The results of our study add to the understanding of the role of the immune microenvironment in DCIS, especially in the HER2+ subtype. The complex interactions between DCIS tumor cells and the local immune system may play a role in breast cancer progression and may be further exploited for immune-based therapeutic strategies.
**2017 San Antonio Breast Cancer Symposium**

**Publication Number:** P2-03-12

**Title:** Incidence and implications for hormone receptor status change pre- and post- neoadjuvant chemotherapy for locally advanced breast cancer: A retrospective single-center cohort study

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**Body:**

**Background:** Hormone receptors (HR) and Human Epidermal Growth Factor Receptor 2 (HER2) status are variables that significantly influence the treatment regimens chosen for each patient (pt). The main objective of this study present was to determine the incidence of HR status change pre- and post- neoadjuvant chemotherapy (NAC) in patients with locally advanced breast cancer (LABC).

**Methods:** A single-centre study was retrospectively conducted at The Ottawa Hospital Cancer Centre looking at a group of 546 pts diagnosed with LABC from 2006-2015. A number of variables were observed including HR and HER2 status, tumor size and grade, regional lymph nodes involvement, presence of DCIS, treatment regimens. 'Positive to Negative' considered pts who were HR and/or HER2 positive pre-NAC and negative post-NAC, 'No Change' represented the pts whose pathology did not change before and after NAC, and 'Negative to Positive' characterized those pts whose HR and/or HER2 pathology was initially negative and became positive post-NAC. Wilcoxon signed-rank test for paired dichotomous variables has been performed in order to estimate statistical significance of changes in status of HR and HER2. To explore possible relationship between HR and HER2 status change and other factors we used Logistic regression method.

**Results:** Of the 386 pts who were examined for Estrogen Receptor (ER) twice, 285 had positive status before surgery, after surgery there were 12 pts (3.11%) with a 'Positive to Negative' change, 362 pts (93.78%) with no change, and 12 pts who represented a 'Negative to Positive' change. 254 of 378 pts, who had two tests for Progesterone Receptor (PR), had positive results at first time, and 29 (7.67%) of those represented a 'Positive to Negative' change, there were 337 (89.15%) with no change, and 12 (3.17%) with a 'Negative to Positive' change. From 387 pts who were tested for HER2 before and after surgery, 105 had initially positive status. Of those, 12 (3.10%) represented a 'Positive to Negative' change, there were 364 (94.06%) with 'No Change' and 11 (2.84%) with a 'Negative to Positive' change. Logistic regression analysis did not reveal any significant factors that can predict changes in receptors’ status. *-Statistically significant changes (p<0.001). The results are comparable with previously published works, which demonstrated changes in IHC status during treatment in metastatic disease (E.Amir, 2011) and a in patients with LABC (Y.Yang, 2013; R.Gahlaut, 2016). Results of comparative meta-analysis will be presented also.

**Conclusions:** Our study shows that a change in HR and HER2 status is common in this population, indicating that close monitoring of biopsy results should be closely compared to surgical pathology specimens to determine a change in biomarkers, and therefore, a potential change in treatment regimen. None of analyzed factors can predict change in status of ER, PR, and HER2 after neoadjuvant treatment. Even though the incidence of change in status is small, the implications for accurate and personalized treatment are important and HR and HER2 testing should be performed as a routine practice on the biopsy and at the time of definitive surgery.
2017 San Antonio Breast Cancer Symposium

Publication Number: P2-03-13

Title: Pathology differentiation during breast conservation surgery using high-frequency ultrasound and peak frequency distribution

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Body: Determining breast tissue pathology in surgical margins is a crucial step in breast conservation surgery (BCS) as this diagnosis decides the need for re-excision. Currently, it can take several days for a pathology laboratory to examine BCS tissue specimens, differentiate between different types of neoplasms (e.g., ALH, LCIS, and ILC), and identify positive margins. Re-excisions cause additional emotional strain, pain, and discomfort for patients, as well as increase medical treatment costs. High-frequency (HF) ultrasound (20-80 MHz) provides a potential new method to discriminate between breast tissue pathologies during BCS. HF ultrasound could also be used to analyze either the surgical cavity in vivo or margin specimens ex vivo. In a 17-patient pilot study that included 14 different pathologies, the HF ultrasonic parameter known as peak density—the number of peaks and valleys in the ultrasonic spectra—displayed a strong correlation to tissue pathology. The objective of this study was to conduct a retrospective analysis of the pilot study data to determine if correlations also existed between the distribution of frequencies of the spectral peaks and valleys with pathology.

The pilot study data were collected from 55 measurements on 34 specimens at the Huntsman Cancer Institute (Salt Lake City, Utah) immediately following BCS and prior to standard pathology preparation and analysis. Through-transmission measurements were acquired with the use of two 50-MHz transducers (Olympus NDT, V358-SU, 6.35-mm active element diameter), a HF square-wave pulser/receiver (UTEX, UT340), and a 500-MHz digital oscilloscope (Hewlett-Packard, HP-54522A). The transmitting transducer was pulsed with a 100-V half-period square wave with a 10-ns width. Waveforms were averaged in the signal acquisition and downloaded onto a computer. Because of the small wavelength of the transmitted ultrasound (30 µm for 50 MHz) in comparison to the size of the active element, the transmitting transducer produced plane-wave pulses which propagated through the tissue to the receiving transducer. Because of the small wavelength and coherent nature of the measurements, the resulting ultrasonic spectra displayed extinction features (peaks and valleys) resulting directly from acoustic wave scattering from cells and nuclei. In this study, the frequencies of the peaks and valleys were plotted as a function of pathology for analysis. The results showed a strong correlation between peak frequency distribution and pathology, particularly in differentiating between malignant and non-malignant pathologies. The peak frequencies for malignant pathologies displayed greater clustering at higher frequencies, whereas the peak frequencies for the non-malignant pathologies displayed greater clustering at lower frequencies. The analyses also verified that the physical mechanism behind the HF ultrasound method is based on acoustic scattering from cells and nuclei, and on the trends arising from the differences between the sizes and morphologies of normal, pre-cancerous, and cancerous cells and nuclei. These results support the use of HF ultrasound as a prospective new method for rapidly distinguishing between different pathologies in breast tissues during BCS.
Body: BACKGROUND: Increasing evidence suggests that epigenetic mechanisms play critical roles in the development of breast cancer. However, precise DNA methylation signatures associated with breast cancer susceptibility remain unknown. We sought to compare DNA methylation changes in the normal breast tissue of women with and without breast cancer to identify patterns of aberrant DNA methylation in women with breast cancer.

METHODS: Samples of normal breast tissue were collected from four cohorts of women: age < 50 years with and without breast cancer, and age ≥ 50 years with and without breast cancer. Normal breast tissue from healthy women was obtained from the Komen Tissue Bank at IU Simon Cancer Center and from women presenting for reduction mammoplasty at Yale New Haven Hospital. Normal breast tissue from women with breast cancer was obtained from patients undergoing adjuvant total mastectomy at Yale Breast Center. DNA was extracted using Qiagen AllPrep Universal kit. Raw data files in idat format were imported to Partek Genomics Suite 6.6 for normalization and differential methylation analysis. Raw intensities were normalized using With Array Normalization (SWAN) method. Principal component analysis (PCA) were performed as quality control. Differentially methylated loci (DML) between control and breast cancer groups were detected when False discovery rate (FDR) < 0.05 and fold change > 1.5. Functional enrichment analysis of genes with DML in the gene body were conducted using METACORE™. Pathways with FDR < 0.05 were selected.

RESULTS: Ninety-three normal breast tissue samples from 89 subjects were analyzed (breast cancer = 40, unaffected = 53). Comparison of DNA methylation patterns between women with and without breast cancer revealed 200 DMLs. The majority of DMLs (186) were hyper-methylated in breast cancer patients, and 48 DMLs locate in enhancers of genes. 170 DMLs locate in 134 genes, enriched in two pathways: (1) Cell adhesion_Endothelial cell contacts by junctional mechanisms, and (2) Neurophysiological process_Constitutive and regulated NMDA receptor trafficking. Genes associated with cell adhesion and cell contacts included: ACTN2, GJA4, GJA7 and MAGI1. Two hyper-methylated loci were found in enhancers of ACTN2. In addition, one hyper-methylated locus in GJA4, one hyper-methylated and one hypo-methylated loci in GJA7, and two hyper-methylated loci in MAGI1 were detected in breast cancer patients. Genes associated with NMDA receptor trafficking include: TPK1, ADCY4 and LIN7C. One and two loci were found in TPK1 and ADCY4, respectively, that were hyper-methylated in normal breast tissue from cancer patients in the gene body, while a hypo-methylated locus in breast cancer patients was identified in LIN7C.

CONCLUSIONS: Comparison of DNA methylation patterns of normal breast tissue from women with and without breast cancer reveal specific mechanistic pathways and genes that are differentially methylated in women with breast cancer. DNA methylation of normal breast tissue deserves further study as a potential biomarker for breast cancer risk stratification and may lend new insight into mechanisms of breast cancer development.
Title: Epigenomic analysis of cancer stem cell (CSC)-enriched triple-negative breast cancer (TNBC) populations reveals gene regulatory circuitry and novel tumor cell vulnerabilities

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Body: Tumor-initiating cells (TICs), also termed cancer stem cells (CSCs) are involved in breast cancer chemoresistance, metastasis and disease progression. To pinpoint tumor cell vulnerabilities and transcriptional drivers of therapeutic relevance, we have characterized the triple negative breast cancer (TNBC) CSC transcriptional landscape using epigenome mapping and nucleosome occupancy determination. We identify a set of transcriptional regulators and signaling mediators that enforce the cancer stem cell state and instruct potential therapeutic strategies.

The basal epithelial marker, integrin-β4 (ITGB4), can be used to stratify mesenchymal-like triple-negative breast cancer (TNBC) cells into populations of low and high tumor-initiating ability in vivo. We used ChIP-seq to measure H3K27ac occupancy and map the transcriptional enhancers in SUM159 cells segregated into ITGB4⇔ (High tumor initiating ability) and ITGB4⇔ (Low tumor initiating ability) populations. Gene-enhancer linking and comparative analysis of enhancer usage revealed an epigenomically defined set of genes that are candidate drivers of the CSC cell state, including GSK3β, DNA-binding transcription factors and cellular adhesion proteins. To further define the chromatin architecture and transcriptional regulatory circuitry that underlies CSC state, we deployed ATAC-seq (Assay for Transposase-Accessible Chromatin with high throughput sequencing) within ITGB4⇔ and ITGB4⇔ populations. By pairing nucleosome occupancy and transcription factor kinetics, we created enhancer-linked transcriptional regulatory circuitry of these tumor-initiating cells.

Together, the isolation of partially mesenchymal ITGB4⇔ CSCs, coupled with enhancer mapping and distillation of transcriptional regulatory circuitry from these cells enable the identification of cancer vulnerabilities and therapeutic opportunities for high-risk patients with TNBC.
Body: Background: In recent years, there has been great interest in developing drugs that modify epigenetic changes as new therapies for breast cancer (BC). There is evidence that aberrant epigenetic inactivation of genes, essential for normal cell growth, is involved in cancer. These modifications are potentially reversible therefore re-activation of genes in response to epigenetic drugs can result in inhibition of tumour growth or sensitisation to other anti-cancer therapies. Epigenetic drugs are in clinical trials for BC but have some drawbacks: while some can be highly effective \textit{in vitro}, their poor stability could compromise their clinical use. Also, high doses required to induce an effect in patients could increase off-site toxicity. As a result there is an urgent need to develop novel systems for the delivery and release of these drugs.

Objectives: Our aim is to develop targeted microbubbles (MBs) to enhance therapeutic effects \textit{in vitro} and \textit{in vivo}. Ultrasound (US)-mediated drug delivery using MBs is proposed as a non-invasive approach for localised drug administration.

Methods: We developed assays using low doses of a DNA methyltransferase inhibitor, called decitabine (DAC), for determining its delivery and effect \textit{in vitro} and \textit{in vivo}. VEGFR2 was assessed as a targeting molecule for therapeutic delivery to tumour vasculature \textit{in vitro} and in a human BC xenograft model. We have generated therapeutic MBs with DAC using a flow-focussing microfluidic platform and conducted \textit{in vitro} studies to observe tumour and tissue responses.

Results: Treatment of triple-negative BC (TNBC) cells with low DAC doses revealed restoration of epigenetically dysregulated tumour suppressor genes. These genes were used as biomarkers for the assessment of DAC effects in a human TNBC mouse model. To evaluate the use of a targeting molecule for drug delivery, specific binding of VEGFR2-targeted MBs on VEGFR2+ve mouse endothelial cells was verified by a flow assay. VEGFR2 expression was assessed longitudinally in xenograft tissue and demonstrated significantly higher VEGFR2 expression in the vasculature of smaller size tumours, indicating the time that delivery of targeted MBs would be most effective. DAC-loaded liposomes or co-administration of DAC and MBs in combination with US using a specifically designed US transducer, were tested \textit{in vitro}. Currently, investigation of the potential of US-triggered drug delivery enhancing efficacy of DAC \textit{in vivo} is being carried out. In addition, combination treatments have been performed \textit{in vitro}, showing increased sensitisation of cells to anthracycline treatment after priming with DAC.

Conclusions: It may be feasible to combine US, MBs and epigenetic therapy in a pre-clinical setting to improve drug efficacy, particularly for drugs that are rapidly degraded within the body. MB delivery may have the potential to reduce the dosage required, thus reducing off-site side effects in patients.
Body: In spite of its high incidence worldwide, only 10-15% of breast cancers can be attributed to hereditary factors, leaving a substantial proportion with unknown causes. Evidence suggests that 26.8% of new breast cancer cases relate to extrinsic factors, such as lifestyle and environmental exposures. Whilst we have increased our knowledge surrounding factors like alcohol, and obesity, little is known in regards to chemicals in the environment (e.g. pesticides) and consumer goods (e.g. plasticisers and preservatives). Previous research has proved inconclusive, with effects only observed at concentrations considerably higher than what has been identified in human tissues. Furthermore, we have not identified a mechanistic link for many compounds. It has been suggested that epigenetics could provide insight, being defined as 'the link between the environment and our genome'. To date, research has identified links between environmental chemicals and breast cancer through the epigenome, however, this has mainly been undertaken using unrepresentative chemical concentrations that are not comparable to observations from human tissues.

The presented research aims to investigate the relationship between low-dose chemical exposures (similar to concentrations found in human tissues) and breast carcinogenesis. To represent the human breast as closely as possible, mammary epithelial cells (MCF-12A) are grown in three-dimensional collagen co-cultures with endothelial (HMMEC) and fibroblast (HMF) cells and exposed to environmentally relevant concentrations of Bisphenol A (BPA) and propylparaben. Utilising Illumina EPIC beadchip arrays, we observe significant alterations to the epigenetic profile of cells in response to low-levels of chemical exposure. In turn, we show that these changes result in modifications to the gene expression of various genes involved in breast cancer initiation and progression. Finally, confocal microscopy confirms abnormalities to acini formation and development indicative of early breast carcinogenesis in response to exposures.

In addition to these results in the MCF-12A cell line, we present preliminary findings from primary patient derived mammary cells obtained from the Breast Cancer Now cell bank (epithelial, fibroblast and myo-epithelial co-cultures). We demonstrate individuals with and without BRCA1 mutations show altered genetic and epigenetic profiles in response to BPA and propylparaben exposures at low levels.

From these findings, we suggest an involvement of BPA and propylparaben exposure in breast cancer risk. Increasing our understanding of chemical contributions to cancer development not only provides opportunities for cancer prevention, but also allows for more effective risk model development. Furthermore, our results improve our understanding of the multifactorial processes that lead to carcinogenesis, aiding in identifying individuals at high risk for screening programmes. Data presented demonstrates a need additional for research in this area and acts as a model for further studies to be undertaken.
Changes of DNA methylation in the development of breast cancer

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Aberrant DNA methylation plays an important role in breast cancer initiation and progression via inactivation of tumor suppressor genes and activation of oncogenes. To better understand the changes of DNA methylation in the development of breast cancer, we analyzed genome-wide DNA methylation DNA data in breast tumor, adjacent normal tissue, and normal breast tissue from healthy women. Using the Illumina MethylationEPIC array, we investigated approximately 850,000 CpG sites. After quality control, 747,803 probes were retained and included the further analysis. To identify DNA methylation markers associated with breast cancer, we performed differential methylation analysis using ChAMP package implemented in Bioconductor. We identified 590 CpG sites that were differentially methylated (P<1X10^-4) between tumor and normal, and 637 between tumor and adjacent normal tissue samples. Further pathway analyses revealed that changes in DNA methylation between tumor and normal breast tissue were mainly involved in neurogenesis and neuro differentiation, oxidative stress, insulin secretion and epithelial morphogenesis; while changes in DNA methylation between tumor and adjacent normal tissue were mainly involved in angiogenesis, cell morphogenesis, and cell component movement. We further validated our findings using RNA-seq data. Our results suggested distinct DNA methylation changes involved in different biological pathways, reflecting potential causal and reactive biological mechanisms in the initiation and the progression of breast cancer.
**Title:** Triple negative breast cancer-specific chromatin conformation links Notch signal to tumor-specific transcriptional program

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**Body:** Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer that is currently incurable with conventional chemotherapy. Some TNBC patients harbor either recurrent somatic gain-of-function mutations or focal amplification in genes coding for Notch receptors. Recent findings suggest that Notch signals, in the absence of Notch mutations are also important to maintain chemotherapy-resistant circulating tumor cells. Importantly, elevated Notch signaling is specifically enriched in the TNBC and is associated with worse survival in this subtype. These findings underscore the importance of Notch signaling in pathogenesis of TNBC and suggest potential new avenue for therapeutic approaches. Despite this, the target genes and oncogenic mechanisms downstream of aberrant Notch signaling are poorly understood. To create a high confidence map of the oncogenic Notch-driven regulome in TNBC, we performed an integrative, comprehensive and functional genome-wide analysis of Notch-driven targets in Notch-mutated TNBC cell lines by combining high-resolution genome-wide chromatin topology data (HiChIP), dynamic mapping of Notch transcriptional complex members and active and repressive chromatin marks (ChIP-seq), as well as evaluation of dynamic transcription (RNAseq) in presence and absence of Notch signals. One important oncogene and critical Notch target in TNBC is MYC. Our integrative analysis identified detailed map of Notch-dependent MYC regulatory network. The TNBC-specific MYC regulatory region, located 5' of MYC, is much more complex compared to other known Notch-driven malignancies and constitutes of a broad domain of active enhancers marked by H3K27ac that are not present in Notch-driven lymphoma and leukemia. Integrative analysis of ChIP-seq and HiChIP revealed multiple enhancers within this broad domain of regulatory elements that are directly bound by the members of Notch transcriptional complex, sensitive to acute changes in Notch activation and that contact MYC promoter via tumor specific chromatin loops. We showed that genetic perturbation of MYC by CRISPR/Cas9 approach was sufficient to kill TNBC cells. Since, MYC is difficult to target pharmacologically an alternative strategy is to use drugs that inhibit factors regulating MYC expression. To this end, elucidating the Notch regulatory landscape, and in particular, the mechanism by which Notch regulates MYC expression in TNBC may reveal new therapeutic strategies to precisely target MYC in Notch-dependent breast cancer.
Whole genome sequencing reveals enrichment of mutations in mucin gene family in breast cancer diagnosed during pregnancy

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Body: Background

Pregnancy is known to modulate breast cancer (BC) risk. Different reproductive behaviors have been shown to impact not only the risk of developing BC but also the phenotypes of these tumors. Breast cancer diagnosed during pregnancy (BCP) is a rare disease but could serve as a good model to understand how pregnancy modulates BC biology. In this project, we aim to interrogate the effect of pregnancy on the biology of BC by performing whole genome sequencing (WGS) using a unique series of BC patients diagnosed during pregnancy (BCP).

Method

Whole genome sequencing was performed for 35 BCP and 20 non-pregnant controls matched for age and stage with available clinic-pathological data. DNA extracted from primary tumor and matched adjacent normal FFPE tissues was assessed using WGS on Illumina HiSeqXTen platform targeting 60x and 30x coverage for tumor and normal DNA respectively. Briefly, 2x150bp paired end sequence data were generated, cleaned, trimmed and aligned to the reference genome (hg19) using bwa-mem. Somatic mutations were detected using Strelka and annotated using SnpEff. Mutational signatures were extracted using deconstructSigs. Differences on mutational profiles between BCP and case controls were assessed using Wilcoxon test for continuous variables and Fisher exact test for categorical variables.

Result

No difference in clinic-pathological features was observed between BCP and control patients. A median of 10084 and 13829 SNVs and of 26 and 21 indels were identified in the BCP and controls respectively, no significant difference between the two groups being observed ($p = 0.703$ and $p = 0.851$). Of interest, a significantly higher number of mutations was found in the BCP as compared to the control group when considering only mutations associated with a deleterious effect (median: 20 vs. 12, $p = 0.027$). As expected, $TP53$ and $PIK3CA$ were the most frequently mutated genes both in BCP and control cases without any significant difference between the groups (34.3% vs. 22.2%, $p = 0.53$ and 20.0% vs. 16.7%, $p = 1$, respectively). Interestingly, there was a significant enrichment of non-silent mutations in the mucin genes family ($MUC2$, $MUC4$, $MUC12$, $MUC16$, $MUC17$, $MUC20$) in the BCP group: 45.7% of BCP vs. 11.1% of control cases had at least one such mutation ($p = 0.015$). A similar significant result (45.7% vs. 23.1%, $p = 0.034$) was found when comparing BCP with BC control cases from the TCGA dataset (selected to have similar age, ER and PR distribution, N = 56). When comparing the distribution of the twelve BC mutational signatures, a borderline significant enrichment with a signature depicting mismatch-repair deficiency (signature 20) was observed in the BCP patients ($p = 0.059$).

Conclusion

This is the first study reporting the mutational landscape of breast cancer diagnosed during pregnancy using WGS. We found that BCP are associated with a higher number of putative driver mutations including mutations in mucin genes, shown to be implicated in tumorigenesis. Furthermore, BCP were enriched with a mismatch-repair deficiency signature. These results could open new avenues for the development of targeted therapeutic approaches for patients diagnosed with breast cancer during pregnancy.
Title: Functional genomic screening identifies ubiquitin-specific protease 11 (USP11) as a novel regulator of ER-alpha transcription in breast cancer

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Body: Approximately 70% of breast cancers overexpress the estrogen receptor α (ERα) and depend on this key transcriptional regulator for growth and differentiation. The discovery of novel mechanisms controlling ERα function represent major advances in our understanding of breast cancer progression and potentially offer attractive new therapeutic opportunities. Here, we investigated the role of deubiquitinating enzymes (DUBs), which act to remove ubiquitin moieties from proteins, in regulating transcriptional activity of ERα in breast cancer.

To identify DUBs involved in the regulation of ERα transcriptional activity, we performed an RNAi loss-of-function screen using a library of shRNA vectors targeting all human DUB genes. The DUB library consisted of pools of four non-overlapping shRNAs targeting all 108 known or putative DUBs (432 shRNAs in total). We found that suppression of a number of DUBs markedly repressed or enhanced the activity of an estrogen-response-element (ERE) luciferase reporter following estradiol (E2) stimulation. Of particular interest, suppression of the BRCA2-associated DUB, USP11, was found to down-regulate ERα transcriptional activity.

Subsequent validation using two individual siRNAs targeted to USP11 revealed a notable reduction in expression of endogenous ERα target genes in the ZR-75-1 cell line, as quantified using qRT-PCR. Further validation was carried out in a HEK293T USP11 knockout cell line, where reduced activity of an ERE-luciferase reporter was detected when compared to wild-type cells. This phenotype was rescued with a USP11 overexpression vector, both in the presence and absence of E2. Furthermore, USP11 expression was found to be upregulated in the estrogen-independent cell line LCC1 when compared to their parental MCF7 cells. Knockdown of USP11 in LCC1 cells resulted in decreased mRNA expression of a panel of ERα target genes, while RNA-seq revealed a downregulation of several putative ERα target genes and a downregulation of many cell cycle-associated proteins. To support the prognostic relevance of USP11, immunohistochemical staining of a breast cancer tissue microarray (103 ER+ patients available for final analysis) was performed. Kaplan-Meier analysis of this cohort revealed a highly significant association between high USP11 expression and poor overall (p=0.030) and breast cancer-specific survival (p=0.041). In silico analysis of publically available breast cancer gene expression datasets further supported an association between high USP11 mRNA levels and poor prognosis. We observed a significant correlation between high expression of USP11 mRNA in ER-positive patients and poor distant metastasis-free survival (HR 2, CI 1.37-2.91, p=0.00023). This correlation was also significant in ER-positive patients who had received tamoxifen only (HR 2.9, CI 1.63-5.15, p=0.00015).

These results suggest a role for USP11 in driving cellular growth and identify USP11 as novel therapeutic target in breast cancer.
Title: Novel driver genetic alterations in MYB-NFIB-negative breast adenoid cystic carcinomas

Body: Introduction: Breast adenoid cystic carcinoma (AdCC) is a rare type of triple-negative breast cancer associated with an indolent clinical behavior. AdCCs provide a clear example of genotypic-phenotypic correlation with the majority harboring the MYB-NFIB fusion gene. In this study, we sought to identify alternative driver genetic alterations in breast AdCCs lacking the MYB-NFIB fusion gene.

Methods: Nucleic acids obtained from four breast AdCCs lacking the MYB-NFIB fusion gene as defined by reverse transcription (RT)-PCR and/or fluorescence in situ hybridization (FISH) were subjected to RNA-sequencing (n=3), whole-genome (n=2) and/or targeted (n=1) massively parallel sequencing. Sequencing data were analyzed using state-of-the-art bioinformatics algorithms, and potential alternative driver genetic alterations were validated using orthogonal sequencing and molecular pathology methods.

Results: RNA-sequencing revealed the presence of MYBL1-ACTN1 or MYBL1-NFIB fusion genes in two breast AdCCs, which were validated by whole-genome sequencing and/or MYBL1 FISH analysis. Both MYBL1 fusion gene-positive cases were found to overexpress MYBL1 as defined by quantitative RT-PCR analysis. In the third MYB-NFIB-negative breast AdCC studied, a high-level MYB gene amplification coupled with overexpression of MYB at the mRNA and protein levels was identified. In the fourth breast AdCC, which expressed high levels of MYB, whole-genome and RNA-sequencing revealed no definite alternative driver alteration, however, a MYBL2 intronic mutation was found in this case, which was associated with high levels of MYBL2 mRNA expression. In this case, single sample gene set enrichment analysis revealed activation of pathways similar to those activated in AdCCs harboring the MYB-NFIB or MYBL1 fusions genes.

Conclusion: We demonstrate that in breast AdCCs lacking the MYB-NFIB fusion gene MYBL1 rearrangements and MYB amplification are likely alternative driver genetic events. Given that activation of MYB/MYBL1 and their downstream targets can be driven by the MYB-NFIB fusion gene, MYBL1 rearrangements, MYB amplification or other yet to be validated mechanisms (e.g. MYBL2 non-coding mutations), our findings further suggest that breast AdCCs constitute a convergent phenotype.
Title: Deregulation of A-to-I RNA editing is associated with poor prognosis in HER2+ breast cancers in the neoALTTO trial

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Body: Background
A-to-I RNA editing, a post-transcriptional modification of the RNA catalyzed by the ADAR family of enzymes, is emerging as a widespread phenomenon in breast cancer (BC). A-to-I RNA editing is more frequent in the highly repetitive Alu regions but can affect both coding and non-coding regions. It has been shown to greatly impact cell functionality. In a recent report, we have shown that A-to-I RNA editing is regulated both by ADAR copy number and type I interferon response (Fumagalli et al. Cell Rep 2015). The main aim of the current study was to investigate the extent and profile of A-to-I RNA editing in HER2+ BC patients (pts) treated in the NeoALTTO trial, and to explore its impact on pathologic complete response (pCR) and survival.

Methods
Aligned RNAseq reads of sufficient quality and quantity were obtained for 252 of the 455 pts enrolled in the study, as described previously (Fumagalli et al. JAMA Oncol 2016). Editing sites from the rediPortal database were assessed. The editing level at a given site was computed by counting the number of Gs and As. Sites with coverage more than 10 were considered for further analyses. Editing in normal tissues was obtained from the GTEx project of the rediPortal database. Tumor infiltrating lymphocytes (TILs) and copy number aberrations were previously reported. Correlations between different parameters were assessed using Spearman correlations (ρ). The Mann-Whitney test was used to relate binary and numerical features. Event-free survival (EFS) analysis was performed using the Cox proportional hazard model.

Results
There was a median of 71470 edited sites per sample. As expected, mean editing per sample correlated with ADAR expression (ρ=59%, p<10^-16) and ADAR copy number (ρ=54%, p<10^-16). It was also correlated with the IFN-gamma driven signature (ρ=22%, p=0.0005), as well as with ESR1 gene expression (ρ=24%, p=0.0002). Neither ADAR expression nor mean editing was correlated with TILs (ρ=-0.5% and p=3%). No relationship between mean editing and pCR or EFS was found. The correlations between editing in NeoALTTO tumor samples and GTEx normal tissues were computed, and the median editing per sample was taken. These median correlations, ranging from 32% to 56%, were not associated with ADAR expression (ρ=-25%, p=6x10^-5) nor mean editing (ρ=8%, p=0.19). Of interest, patients whose tumors showed low correlation with editing in normal tissues were associated with poor EFS (ρ=0.028, HR=0.56 to 0.96) suggesting that deregulation of RNA editing may impact disease progression and outcome. Similar results were obtained when the correlations were assessed between tumor samples instead of between tumor and normal samples (ρ between the two median editing: 76%; p-value survival: 0.013). The median correlations were not predictive for pCR (ρ=0.44). There was no interaction between editing and treatment arm.

Conclusions
Our study shows for the first time that deregulated RNA editing, as compared to editing in normal tissues, is a widespread phenomenon in HER2+ BC patients treated in the NeoALTTO trial and is associated with poor outcome. These results may provide new perspectives for the treatment of HER2+ disease by developing therapies targeting RNA editing.
Title: Genetic heterogeneity as a risk assessment parameter in breast cancer

Body: Cancer is a disease of clonal evolution, and intra-tumor heterogeneity provides the fuel for that evolution. Unfortunately, that heterogeneity poses a challenge for sampling and prognosis, as different biopsies may sample different clones, which may not be relevant to the future behavior of the tumor. However, because heterogeneity helps to drive clonal evolution, measurements of heterogeneity itself may be prognostic. We hypothesized that ductal carcinoma in situ (DCIS) with more clonal heterogeneity would be more likely to progress to invasion and metastasis.

In order to investigate cancer heterogeneity, we have developed new bioinformatics methods that allow us to analyze small amounts of degraded DNA (20ng) extracted from FFPE samples, at multiple regions. Using our method, we investigated the exomes of pairs of DCIS samples taken at 10 mm distances in the same neoplasm. We identified the mutations present in each sample and we evaluated the heterogeneity level between the pairs of samples. Finally, we compared the heterogeneity levels of patients with pure DCIS versus DCIS patients with invasive features.

We found a statistically significant different level of genetic heterogeneity between pure DCIS and invasive DCIS patients. Notably, the DCIS that has adjacent invasive components have a larger number of private mutations than the patients with DCIS alone (mean 31 vs 16 mutations, Mann-Whitney p=0.023), suggesting that heterogeneity may have a relevant role on cancer progression.

This finding supports our ongoing work to further elucidate the relationship between clonal heterogeneity and invasive progression in breast cancer.
Title: Mitochondrial gene fusions and their putative roles in breast cancer

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Body: Background: Several mitochondrial DNA (mtDNA) mutations have been identified in human cancer, and suppression of mitochondrial respiratory gene expression has been reported. mtDNA has limited protective mechanisms due to lack of histones and is more susceptible to genomic aberration. While nuclear gene fusions are known to play an important role in tumorigenesis, the role of mitochondrial gene fusions in tumorigenesis and human disease has not been defined. Given the importance of nuclear gene fusions in human cancer, we sought to define the role of mtDNA gene fusions in the clinical behavior of breast tumors and their response to therapy.

Methods: RNA sequencing was performed on pre and post treatment breast cancer samples obtained from neoadjuvant clinical trials BrUOG 211A/211B. There were 73 baseline and 61 post-treatment samples, with 54 samples at both time points. Patients were given a run-in dose of bevacizumab (B), nab-paclitaxel (N) or trastuzumab (T), followed by combination biologic/chemotherapy (HER2- with B/carboplatin/N; HER2+ with T/carboplatin/N). We detected gene fusions using TopHat-Fusion and defuse based on RNA derived from biopsy pairs obtained pre/post 10-day exposure to run-in monotherapy. Results: In this cohort, 385 mtDNA gene fusions were detected in baseline and 344 in post-treatment samples, with an average of 4.6 and 4 mtDNA gene fusions, respectively, per patient, with the following observations:

(1) A number of fusions had recurrent increase from baseline to post-treatment, e.g. MT-ATP6:MT-ND2 (N = 9), MT-ND6:MT-CO1 (N = 6), MT-ND6:MT-CO1 (N = 10), and MT-ND4:MT-ATP6 (N = 9)

(2) Gene fusions MT-ND6:MT-ATP8, MT-CYB:MT-ND2 and MT-ND6:MT-ND1 were observed respectively in 9, 5 and 4 baseline samples but not in their post treatment equivalent.

(3) MT-ND6 had seven gene fusion partners that appeared in fusions that were most abundant among baseline and post-treatment samples (MT-ND4 (N = 39), MT-ND2 (N = 37) and MT-ATP6 (N = 36)).

Of note, MT-CO1 and MT-CO2 both encode the enzyme cyclooxygenase, with MT-CO2 being the inducible isoform that activates inflammatory response and promotes mammary carcinogenesis. MT-ATP6 and MT-ATP8 encode the ATP synthase subunits 6 and 8, and novel mutations have been identified in these genes in breast cancer patients. MT-ND1/2/4/6 encode the subunits of NADH dehydrogenase (Complex I) which is the largest of the respiratory complexes. Polymorphisms in these genes are associated with breast cancer risk and progression.

Conclusions: While several studies have examined the presence of mtDNA mutations in breast cancer, the role of mitochondrial gene fusions is unknown. Many of the mtDNA genes involved in fusions have been shown to play a role in breast tumorigenesis and the clinical behavior of breast tumors. We observed mitochondrial fusions in our cohort of breast tumors treated with preoperative therapy, some of which were seen in pre and post treatment samples suggesting a potential role in resistance to therapy. Further study of mtDNA fusions in breast cancer is needed as this may have important implications for treatment of this disease.
Title: Whole exome sequencing of extreme responders reveals low mutation burden in metastatic breast cancer

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Body: Background: Extreme responders toanticancer therapy are rarely encountered in the treatment of advanced breast cancer patients, but their treatment response have not been investigated on the whole exome level. We performed whole exome analysis to characterize genomic landscape of extreme responders in metastatic breast cancer patients.

Methods: Clinical samples were obtained from patients who showed exceptional response to anti-HER2 therapy or hormonal therapy. Non-responders were selected among those who did not respond. Matched breast tumor tissue (somatic DNA) and blood samples (germline DNA) were collected from a total of 18 responders (12 ER+, 6 HER2+) and 8 non-responders (6 ER+, 1 HER2+, 1 TNBC). Whole exome sequencing using Illumina HiSeq2500 was performed on the 26 patients (52 samples). Somatic single nucleotide variants (SNVs), indels and copy number variants (CNVs) were identified for each patient genome. Group specific somatic variants and mutation burden were statistically analyzed.

Findings: Cancer exomes were characterized by 1,455 somatic single-nucleotide variants (1,327 missense, 80 nonsense, 36 splice-site, 12 start/stop-lost), 149 insertions/deletions (108 frameshift, 41 inframe), with a median of 1 mutations per Mb (0.2 to 2.7 mutations per Mb) in all patients. Responders harbored a significantly lower non-synonymous mutation burden than non-responders (median, 27 vs. 90.5, \( P = 0.01 \)), and copy number variation burden was also lower (median 23 vs. 31, \( P = 0.14 \)). Multivariate analyses of factors influencing progression-free survival showed that high mutation burden and visceral metastases were significantly related with progression.

Interpretation: Extreme responders of metastatic breast cancer are characterized by low nonsynonymous mutational burden.
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Title: Mucinous breast carcinomas: A genomically distinct subtype of estrogen receptor-positive invasive breast cancers

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Body: Introduction: Mucinous carcinoma of the breast (MCB) is a rare histologic form of estrogen receptor (ER)-positive invasive carcinoma, accounting for up to 2% of breast cancers. MCBs are characterized by clusters of tumor cells floating in lakes of extracellular mucin, and are classified into mucinous A (paucicellular) and mucinous B (hypercellular) subtypes. Some MCBs are found admixed with invasive ductal carcinoma components, and then classified as mixed MCBs. The aims of this study were to determine the repertoire of somatic mutations of MCBs and to ascertain whether these genetic alterations are distinct from those identified in common forms of ER+/HER2- invasive breast cancers (IBCs). We also sought to determine whether the mucinous and ductal components of mixed MCBs would be clonally related.

Materials and methods: Thirty MCBs including 25 pure MCBs (n=13 mucinous A, n=12 mucinous B) and five mixed MCBs were microdissected and subjected to whole exome sequencing. Each tumor component of mixed cases was microdissected and analyzed separately. Somatic mutations, copy number alterations and mutational signatures were defined using state-of-the-art bioinformatics methods. The mutational repertoire of MCBs was compared with that of ER+/HER2- IBCs (n=240) from The Cancer Genome Atlas (TCGA) breast cancer study.

Results: The genes most frequently mutated in MCBs were GATA3 (27%, 8/30, all frameshift mutations), KMT2C (13%, 4/30) and MAP3K1 (10%, 3/30). No significant differences were identified in single gene comparisons between mucinous A and mucinous B MCBs or between pure MCBs and the mucinous component of mixed MCBs (Fisher’s exact tests, p>0.05). As compared to common forms of ER+/HER2- IBC, MCBs had a lower frequency of PIK3CA mutations (7% vs 42%, p<0.001) and a higher frequency of GATA3 mutations (27% vs 12%, p=0.04). Mucinous B MCBs had a higher frequency of KMT2C mutations than ER+/HER2- IBCs (25% vs 6%, p=0.04). Most MCBs displayed the mutational signature 1 (aging-related; 20/30, 67%), and no differences in the frequency of specific mutational signatures according to the type of MCBs were observed. Concurrent 1q gains and 16q losses, which are the hallmark genetic alterations of low-grade ER+/HER2- breast cancers, were not observed in pure MCBs, but were found in three of the five mixed MCBs analyzed. The mucinous and ductal components of all five mixed MCBs shared a median of 58% of somatic mutations (range 42%-64%), including clonal GATA3 frameshift mutations in two of them, as well as a similar pattern of copy number alterations, supporting their clonal relatedness. Additional somatic mutations found to be restricted to the ductal or mucinous components of all mixed MCBs analyzed were identified, including clonal missense mutations in PIK3C2B and PIK3R2 in the ductal component of one case, and a PIK3R5 missense mutation in the mucinous component of another case.

Conclusions: The repertoire of somatic mutations in MCBs is distinct from that of common forms of ER+/HER2- IBCs. These differences include the lack of concurrent 1q gains/16q losses, a lower frequency of PIK3CA mutations and a higher frequency of GATA3 mutations in pure MCBs.
Title: Genomic differences and impact on clinical outcomes in patients with pre- and post-menopausal metastatic breast cancer

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Body: Background
Breast cancer accounts for 15% of all new cancers and 7% of all cancer-related deaths. Women diagnosed before the age of 40 years have considerably worse survival rates making this variable an independent risk factor for adverse prognosis. Furthermore, the management of ER+ breast cancer is particularly different between pre- and post-menopausal women. However, there is a paucity of data comparing the genomic landscape of pre- and post-menopausal ER+ breast cancer, particularly differences in actionable alterations. Here, we compare the incidence of mutations in 91 actionable genes in pre-menopausal and post-menopausal metastatic ER+/HER2- breast cancer patients.

Methods
We identified patients with metastatic ER+/HER2- breast cancer who had tumor genotyping testing by Snapshot-NGS assay via multiplex polymerase chain reaction (PCR) technology called Anchored Multiplex PCR, utilizing the ArcherDx platform and Illumina NextSeq next generation sequencing (NGS), for single nucleotide variant (SNV) and insertion/deletion (indel) detection in genomic DNA from formalin-fixed, paraffin-embedded (FFPE) tissue. An ensemble variant calling approach was utilized incorporating MuTect1, LoFreq, GATK, and a laboratory developed hotspot caller. All sequencing and variant calling was performed at the Massachusetts General Hospital Center for Integrated Diagnostics. Demographic data including menopausal status at the time of metastatic diagnosis was determined based on retrospective chart review.

Results
We analyzed the tumor genotyping results from 34 pre- and 98 post-menopausal patients with ER+/HER2- metastatic breast cancer. Pre- and post-menopausal groups were determined to have a median age of 47 and 64, and premenopausal women were more likely to have ER+/PR- disease. While there was no statistically significant difference in the rate of mutation in any gene after multiple testing correction, PIK3CA (0.35 vs. 0.24, OR=1.8, P=0.2), PTEN (0.12 vs. 0.06, OR=2.23, P=0.5), and ESR1 (0.09 vs 0.03, OR=3.4, P=0.45) mutations were skewed towards the post-menopausal group. Conversely, NOTCH1 (0.06 vs 0.02, OR=2.9, P=0.27) and MSH6 (0.03 vs 0, OR=inf, P=0.26) mutations were skewed towards the pre-menopausal group. The impact of these molecular alterations on clinical outcomes will be presented at the symposium.

Conclusions
Our findings highlight key differences in the genomic landscape of actionable genes between pre- and post-menopausal metastatic breast cancer, which may serve as markers for understanding and predicting disease prognosis. Furthermore, our data aim to clarify the utility of routine clinical sequencing panels in pre-menopausal metastatic breast cancer.
Title: ASCO 2013 HER2-equivocal breast cancers CGH array analysis

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Body: Background
The status of human epidermal growth factor receptor 2 (HER2, ERBB2) determines breast cancer patients HER2-targeted therapy eligibility. New international guidelines on HER2 determination in breast cancers have been proposed by ASCO/CAP in 2013. HER2 level of amplification is associated to the quality of the anti-HER2 therapy response. The answer to anti-HER2 therapy for equivocal cases remains debated.

Objectives
We first evaluated the impact of the 2013 ASCO/CAP updated guidelines on HER2 fluorescence in situ hybridization (FISH) classification in breast cancers with an equivocal (Score 2+) immunohistochemistry (IHC) result from 2008 to 2014 in our institution. We then investigated genome-wide copy number alterations in breast cancer patients with HER2-equivocal result by both IHC and FISH.

Methods
We reviewed all breast cancers cases analysed by FISH for HER2 status determination between 2008 and 2014 following 2007 ASCO guidelines and reassessed them according to 2013 guidelines. Breast cancers with equivocal HER2 status by both IHC and FISH (49 cases) according to 2013 guidelines were subsequently analysed by Agilent 60-mer oligonucleotide microarrays for array-based comparative genomic hybridization (aCGH).

Results
When applying the 2013 ASCO guidelines, 141 cases (7.2%) out of 1970 cases analysed by FISH had a modified HER2 status. Only five (3.5%) in the HER2-negative category defined with the 2007 ASCO guidelines changed into a positive status. The equivocal category increased from 16 (0.8%) to 119 (6.1%) cases. Out of the 49 HER2-equivocal cases identified by both IHC and FISH (ASCO 2013 guidelines), aCGH showed that 20 cases (40.8%) presented a large copy number gain of chromosome 17, 12 cases (24.5%) had a segmental copy number gains including HER2 and five cases only (10.2%) showed HER2 amplification.

Conclusion
The 2013 ASCO/CAP guidelines increased the number of equivocal results and raised an uncertainty about the most appropriate clinical management of these patients. At the genomic level, HER2-equivocal cases corresponded mostly to chromosome 17 polysomy and / or segmental copy number gains. Genomic copy number analysis may be useful in reclassifying equivocal cases which enabled proper patient selection for HER2-targeted therapy.
Title: CYP19A1 gene Rs1008805 polymorphism is related to the prognosis of early breast cancer

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Body: Background: Aromatase, encoded by CYP19A1 gene, is a rate-limiting enzyme in the conversion of androgens into estrogens. Given the critical role of CYP19A1 gene in estrogen synthesis, the potential impact of genetic polymorphisms in CYP19A1 gene on the survival of breast cancer deserves further study.

Methods: Rs1008805 polymorphism in CYP19A1 gene were genotyped on 406 Chinese Han women with stage I-II and operable stage III breast cancer. Associations were explored between rs1008805 genotypes and disease-free survival (DFS).

Results: Totally, there were 200 (49.3%) patients with AA genotype, 169 (41.6%) with AG variant, and 37 (9.1%) carrying GG variant. No significant differences were found in DFS or 5-year survival rate among the whole population with these three genotypes. However, in postmenopausal women, rs1008805 genotypes were significantly related to DFS and 5-years DFS rate (AA versus AG versus GG: 63.1 months versus 54.3 months versus 13.7 months; 48.6% versus 46.0% versus 14.3%; \( P = 0.015 \)). In addition, women with GG variant had a poorer DFS, 5-years DFS rate when compared with those carrying AG or AA genotype (GG versus AG or AA: 13.7 months versus 56.3 months; 0% versus 52.1%; HR, 2.462; 95% CI, 1.310-4.628; \( P = 0.004 \)). Being adjusted by patients features in multivariate analyses, GG genotype remained an independent prognostic factor for DFS (HR, 2.706; 95% CI, 1.393-5.257; \( P = 0.003 \)). Whereas, premenopausal women with the homozygous minor allele had a marginally improved DFS, 5-years DFS rate when compared with those carrying the common allele (GG versus AG or AA: 87.0 months versus 48.7 months; 60.3% versus 42.7%; HR, 0.544; 95% CI, 0.295-1.003; \( P = 0.051 \)). However, there was no relationship between GG genotype and DFS or 5-year DFS when adjusted by patients features in multivariate analyses.

Conclusions: The present study demonstrated that homozygous minor allele of rs1008805 SNP in CYP19A1 gene was significantly related to a worse DFS or 5-year DFS in postmenopausal women with early breast cancer. This founding is novel, if confirmed, CYP19A1 rs1008805 genotypes may turned to be a prognostic biomarker for early breast cancer.
Title: Detection of splice variants related to endocrine resistant hormone receptor-positive breast cancer

Han-Byoel Lee¹, Min-Su Kim², Jiyoung Rhu¹, Jung Hyun Park¹, Kyung Eun Kim¹, Young Wook Ju¹, Eun-Shin Lee¹, Hyeong-Gon Moon¹,², Dong-Young Noh¹,³, Sun Kim⁴ and Wonshik Han¹,³. ¹Seoul National University College of Medicine, Seoul, Korea; ²Interdisciplinary Program in Bioinformatics, Seoul National University, Seoul, Korea; ³Cancer Research Institute, Seoul National University, Seoul, Korea and ⁴Interdisciplinary Program in Bioinformatics, and Bioinformatics Institute, Seoul National University, Seoul, Korea.

Body: Introduction: Estrogen receptor is expressed in 75% of breast cancers and is related to a relatively indolent phenotype. Yet, up to 25% of these tumors develop resistance to endocrine therapy. Alternative splicing events are observed in almost every hallmarks of cancer, implying that dysregulation of splicing and cancer progression are closely related. The purpose of this study was to detect splice variants related to endocrine resistance in hormone receptor(HR)-positive breast cancer.

Methods: RNA sequencing data of 455 HR-positive patients with documented endocrine treatment from The Cancer Genome Atlas (TCGA) database was used for analysis. Splice variants of 96 ESR1 pathway-related genes were detected using a data-mining algorithm recognizing spliceomic heterogeneity. A differential analysis of splice variants between 48 endocrine therapy-resistant and 407 endocrine therapy-responsive patients was performed to discover isoforms frequently detected in endocrine-resistant tumors. Isoforms related to endocrine resistance was further analyzed using whole transcriptome sequencing data from 59 HR-positive invasive breast cancer patients (24 endocrine therapy-resistant, 35 endocrine therapy-responsive who underwent operation at Seoul National University Hospital.

Results: Of 96 ESR1 pathway-related genes, 17 genes showed statistically different splice variant isoforms frequencies (AKT1, ATF2, ATF4, CALM2, CALM3, CREB1, EGFR, ESR1, ESR2, GRM1, HRAS, HSP90AA1, OPRM1, PIK3R3, PRKACB, SHC1, and SHC4). A differential analysis of these isoforms using SNUH data confirmed a predominant isoform of HRAS (64.47% vs 57.14%, p-value 0.0037) and a minor isoform of SHC1 (25.53% vs 32.33%, p-value 0.0456) in endocrine therapy-resistant HR-positive patients. In the same analysis using HR-negative patients, the mean isoform percentage was similar between patients with distant recurrence and no recurrence.

Potential Spliceomic Signatures Reproduced From Seoul National University Hospital Data

<table>
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<tr>
<th>Gene</th>
<th>Hormone Receptor Positive</th>
<th>Hormone Receptor negative</th>
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<td>Mean Isoform % in</td>
<td>Mean Isoform % in</td>
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<td>Responsive Specimens</td>
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Conclusions: Phenotype-specific splice variants can be detected using transcriptome sequencing data. Splice variants in HRAS and SHC1 are potential spliceomic signatures that may be used to predict endocrine therapy-resistant breast cancer. Further investigation is warranted to explore the biological role of these isoforms and identify the role of splice variants as a biomarker for endocrine resistance.
Young breast cancer patients (<40 yo) have unfavorable subtypes, higher stage and worse survival

Jessica S Young¹, Tsutomu Kawaguchi¹, Li Yan¹, Qianya Qi¹, Song Liu¹ and Kazuaki Takabe¹. ¹Roswell Park Cancer Institute, Buffalo, NY.

BACKGROUND:
Over the last 40 years, the incidence of breast cancer in young women in the U.S. has been relatively low and stable, but the absolute number of young women with breast cancer is increasing because of the growing population. Some epidemiological studies have shown that breast cancer diagnosed before age 40 have significantly worse overall 5-year survival. Disease free survival is also inferior in young women, and they have more aggressive cancers in general. This study aims to validate these findings using genomic analysis of large databases.

MATERIALS AND METHODS:
The Cancer Genome Atlas (TCGA; n= 1095) and the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC; n=1894) were used for analysis. We divided the database into the Young (<40 yo) and Non-Young (>40 yo) cohorts, based on age at diagnosis. The following analysis will give the TCGA and METABRIC results in each category, respectively.

RESULTS:
There were 8.9% (98) and 6% (116) patients who were found to be Young. In the Young cohort, 69.5% (64) and 37.9% (44) were ER(+), whereas 77.9% (742) and 79.5% (1415) in the Non-Young cohort were ER(+). Further, 60.8% (56) and 31.9% (37) were PR(+) in the Young cohort, compared to 68.4% (641) and 54.4% (972) in the Non-Young cohort. Her2(+) cancers were noted in 22.2% (12) and 25% (29) in the Young cohort, whereas 22.6% (152) and 11.6% (207) in the Non-Young cohort. Our group developed a pipeline to calculate PAM50 from the RNA-Seq dataset. Utilizing this calculated PAM50 in TCGA, we found that there were less Luminal A and B patients in the Young cohort, 41.6% (42) and 17.8% (18) compared to 49.7% (377) and 22.9% (174) in the Non-Young cohort. This was also the case in METABRIC where 17.2% (20) and 9.5% (11) were Luminal A and B, compared to 36.9% (659) and 25.2% (450) in the Non-Young group. In contrast, there were more basal-like subtypes in the Young group, 17.8% (18) and 28.4% (33), as compared to the Non-Young group, 16.1% (122) and 9.3% (166). These results agree with previous epidemiological studies that showed that hormone receptor positive tumors increase and basal-like subtypes decrease with age. The number of Stage I patients was lower in Young patients 13.5% (13) and 25.3% (22), than in Non-Young patients 17.3% (169) and 34.4% (453). Similarly, there were less Stage II patients in the Young 54.2% (52) and 58.6% (51) compared to 58.3% (569) and 56.9% (749) in the Non-Young. This reverses in Stage III where the incidence is increased in the Young at 31.2% (30) and 16.1% (14) compared to 22.4% (219) and 7.7% (101) in the Non-Young. Young patients had a lower median disease-free survival than Non-Young patients (NA vs 214.7 mo, p=0.027); however, there was no statistical significance in median survival. Young patients had a lower median disease-specific survival than non-young patients of 221.1 months vs 282.6 months (p=0.00123) in METABRIC.

CONCLUSION: We used large datasets to examine survival in very young breast cancer patients (<40 yo) vs older patients and found that young patients are likely to have unfavorable subtypes, higher stage, and a lower overall survival and DFS as compared to their older counterparts. Further analysis with genomics is needed.
2017 San Antonio Breast Cancer Symposium

Publication Number: P2-05-15

Title: Oncotype DX Breast Recurrence Score®: Real-life utilization among node positive patients in Ontario

Roy Richardson¹, Amanda Macchiusi¹, Ashwini Bhasin¹ and Claire Takizawa². ¹Genomic Health Canada, Canada and ²Genomic Health International SARL, Switzerland.

Body: Aims: The 21-gene Oncotype DX (ODX) Recurrence Score (RS) has been extensively validated to predict the risk of distant recurrence and the magnitude of response to hormone and chemotherapy in patients with ER+ N0 and N+ (up to 3 positive nodes) HER2- early stage breast cancer. Outcomes data from multiple large studies further confirm the assays clinical validity and utility. The test is currently reimbursed in Ontario for N0 but not for N1 patients. In 2016, a market access program has been initiated to address lack of data in current clinical practice in Ontario among N1 patients. It collects data assessing the real-life use of the test in Ontario and its impact on treatment decision. This analysis presents the interim analysis until end of May 2017.

Methods: The program allows for prospective data collection in key breast cancer centers in Ontario. Through an online dedicated platform patient data are collected including classical pathological and clinical parameters (e.g. histology, tumor grade and size, ER, PR, and HER2 status), patients age, ODX Recurrence Score results and recommended treatment both before and after the test results have been reported.

Results: A total of 7 qualified breast cancer centers or network of centers participated in the program and collected 90 cases so far. Study results demonstrated that ODX is used among a wide variety of patients profiles: 28% G1, 58% G2 and 14% of G3, 18% pre-, 12% peri- & 69% are post-menopausal, 20% are aged 40-49, 24% are 50-59, 29% are 60-69 and 26% are 70 and older, 3% tumor are <1cm, 38% are 1-<2cm, 52% are 2-<5cm and 7% tumor size are >5cm. RS distribution is the following: <18 (67%), 18-30 (30%) and >30 (3%).

In addition, pre-ODX 90% of patients had a treatment recommendation for chemo-hormonotherapy (CT-HT). Post-testing, the number of patients recommended CT-HT decreased to 36%, highlighting that the test reduces unnecessary use of CT. In fact, the ODX led to an overall 54% net reduction in chemotherapy.

Conclusions: This real-life survey confirms that ODX provides critical information beyond clinical and pathological criteria. The assay changes treatment decisions among N+ patients in Ontario, sparing CT which could result in potential savings to the healthcare system.
Title: Molecular characterization of human malignant phyllodes tumors reveals potential targeted approaches

Hyeong-Gon Moon¹, Jihui Yun¹, Bok Sil Hong¹, Eunshin Lee¹, Han-Byoel Lee¹, Wonshik Han¹, Jong-Il Kim¹, Dong-Young Noh¹, Woohang Heo¹, Saem Hur¹, Wonyoung Kang² and Charles Lee². ¹Seoul National University College of Medicine, Seoul, Korea and ²Jackson Laboratory, Farmington, CT.

Body: Malignant phyllodes tumor (MPT) which belong to the fibroepithelial neoplasm spectrum is a rare type of breast malignancy, and currently there is no effective targeted approach available for MPT. In this study, we tried to identify key genomic alterations and biologic pathways in MPT by whole exome and RNA sequencing of nine MPT tissues. Whole exome sequencing revealed somatic alterations in EGFR, MED12, PIK3CA, PIK3R1, PDGFRA, PDGFRB, PTEN, and TP53. Transcriptome sequencing showed dysregulation of ECM-receptor interaction, focal adhesion, and PI3K-Akt signaling in MPTs when compared to normal breast or invasive breast cancer tissues. Based on the transcriptome profiles, the MPTs were classified into two subtypes; fibrous subtype with upregulation of stromal genes such as collagens and epithelial subtype with upregulation of E-cadherin and Claudins. The molecular classification of fibrous and epithelial subtypes was validated in 28 paraffin-embedded MPT tissues. The fibrous subtype showed higher mitotic index and increased risk for recurrence when compared to the epithelial subtype. We established a patient-derived xenograft model from one fibrous subtype MPT which harbored somatic mutation in PIK3R1 and PDGFRB. In that model, targeted treatment against PIK3CA/mTOR and PDGFR pathways effectively suppressed the tumor growth in vivo. Our data provide insights on the biologic understanding of MPT and suggest a clinically relevant molecular classification. Furthermore, we show that developing effective targeted approaches in MPT can be possible with genomic profiles and patient-derived xenograft models. The clinical efficacy of targeting PDGFR and PIK3CA/mTOR pathways in MPT should be tested in future clinical trials.
Title: Breast cancer HER2 epigenetic intratumoral heterogeneity results from lack of HER2 protein translation

Hiroaki Nitta¹, Rie Horii², Adrian Murillo¹, Bryce Portier¹ and Futoshi Akiyama². ¹Ventana Medical Systems, Inc., Tucson, AZ and ²Japanese Foundation for Cancer Research, Tokyo, Japan.

Body: Research objective
Previously, we reported the negative correlation between pathological complete response (pCR) and HER2 positive breast cancer exhibiting amplified HER2 gene tumor cells without HER2 protein overexpression (HER2 epigenetic intratumoral heterogeneity) among trastuzumab-based neoadjuvant chemotherapy treated patients. Our objective in this study was to elucidate if tumor cells with HER2 epigenetic intratumoral heterogeneity express HER2 RNA using a HER2 RNA in situ hybridization (ISH) method.

Materials and methods
Formalin-fixed, paraffin-embedded (FFPE) sections of breast cancer biopsy samples were investigated for HER2 RNA expression at the individual cell level using a HER2 RNA ISH assay. RNA preservation in tissue sections was examined using a peptidylprolyl isomerase B (PPIB) RNA ISH assay.

Three groups of cases were examined:
1) HER2 negative breast cancer cases (HER2 RNA ISH negative control group).
2) HER2 positive breast cancer cases with homogeneous HER2 positive tumor cells (HER2 RNA ISH positive control; pCR group)
3) HER2 positive breast cancer cases with HER2 epigenetic intratumoral heterogeneity (a mixture of HER2 gene and protein positive tumor cells and HER2 gene positive tumor cells without HER2 protein expression; incomplete response group)

Consecutive sections of HER2 RNA ISH slides were stained for HER2 gene and protein concurrently on the same tissue section using HER2 gene-protein assay (GPA) which is a combination of FDA-approved HER2 immunohistochemical (HER2 protein) and HER2 dual ISH (HER2 gene and chromosome 17 centromere) assays. Analyses of HER2 RNA expression in individual cells was microscopically evaluated and matched to HER2 GPA slides.

Results
RNA preservation was confirmed in tissue sections of all three groups by a PPIB RNA ISH assay. Tumor cells of HER2 negative breast cancer cases (negative control group) lacked HER2 RNA ISH signal while HER2 gene and protein positive tumor cells of homogeneous breast cancer cases (positive control group) demonstrated high HER2 RNA expression levels. HER2 gene and protein positive tumor cells of HER2 positive intratumoral heterogeneity cases showed high HER2 RNA expression. However, amplified HER2 gene breast cancer cells without HER2 protein overexpression of HER2 positive intratumoral heterogeneity cases also showed high levels of HER2 RNA expression. Thus, revealing in cases with intratumoral heterogeneity, transcription of HER2 RNA occurs, but translation of HER2 protein is altered by some mechanism(s) in tumor cells.

Conclusions
Transcription of HER2 RNA was observed in breast tumor cells with amplified HER2 gene but absence of HER2 protein overexpression (HER2 epigenetic intratumoral heterogeneity) of patients who showed incomplete response to neoadjuvant trastuzumab therapy. Our study suggests that inconsistent HER2 protein translation in breast cancer with HER2 epigenetic heterogeneity might be the primary resistance mechanism to trastuzumab-based neoadjuvant chemotherapy.
Title: Clinicopathologic characteristics and genomic essence analyses revealed estrogen receptor positive, progesterone receptor negative and human epidermal growth factor receptor 2 negative breast cancer to be more basal-like and endocrine resistant

Xiyu Liu¹,²,³, Ding Ma¹,²,³, Yi-Zhou Jiang¹,²,³, Yi-Rong Liu¹,²,³, Ke-Da Yu¹,²,³ and Zhi-Ming Shao¹,²,³. ¹Fudan University Shanghai Cancer Center, Shanghai, China; ²Cancer Institute, Fudan University Shanghai Cancer Center, Shanghai, China and ³Shanghai Medical College, Fudan University, Shanghai, China.

Body: Background: Estrogen receptor positive/ progesterone receptor negative/ human epidermal growth factor receptor 2 negative (ER+/PR-/HER2-) tumors define a distinct subtype of ER+ breast cancer, characterized by poor response to endocrine therapy and poor prognosis. The genomic landscape of ER+/PR-/HER2- tumor has yet to be systematically studied, and driver events for tumor progression and endocrine resistance with loss of PR expression are yet to be identified. Methods: We assessed 13,410 ER+/PR-/HER2- patients from Surveillance, Epidemiology, and End Results (SEER), the Cancer Genome Atlas (TCGA) and Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) for clinicopathologic characteristics, survival and genomic analyses. Patients were divided into endocrine-resistant and endocrine-sensitive groups using an endocrine therapy sensitivity score \((0.8*\text{ER}+1.2*\text{PGR}+\text{BCL2}+\text{SCUBE2})/4\), and we further explored genomic events enriched in the endocrine-resistant group. Results: Clinicopathologic features and survival outcomes of ER+/PR-/HER2- patients were worse than ER+/PR+/HER2- in SEER and METABRIC cohort. The genomic landscape of ER+/PR-/HER2- tumors revealed them to have more aggressive properties, like a higher percentage of luminal B (29-35%) and basal-like (4-16%) subtypes; more intClust subtypes with high genomic instability (intClust1, 6); more TP53 mutation and less PIK3CA mutation; and more copy number variation events. ER+/PR-/HER2- tumors have more focal amplification events, including 8p11.23 (ZNF703) and 17q23.1 (RPS6KB1) and more focal deletion events, such as 17q21.31 (BRCA1) and 14q24.3 (MLH3). Pathifier pathway analyses showed that up-regulated pathways in ER+/PR-/HER2-tumors included those associated with biosynthesis, metabolism, drug resistance and DNA replications (FDR<0.1). Endocrine sensitivity score for ER+/PR-/HER2- group was significantly lower than ER+/PR+/HER2- group. When split by 1st quartile of endocrine sensitivity score, up to 72.7% of ER+/PR-/HER2- tumors were grouped into the endocrine-resistant group. Mutant-allele tumor heterogeneity (MATH) score is significantly lower in the endocrine-resistant group. Several focal SCNA events were more common in the endocrine-resistant group (P<0.05), including 8q24.21 amplification with only the MYC gene in the “peak” region. Pathways unregulated in the endocrine-resistant group of ER+/PR-/HER2- tumor included MYC pathway and mTORC1 signaling (FDR<0.25). Conclusion: ER+/PR-/HER2- breast cancer was a heterogeneous tumor group and featured more basal-like and luminal B characteristics. The ER+/PR-/HER2- tumors are relatively resistant to endocrine therapy, and MYC pathway enrichment and corresponding 8q24.21 amplification might be one of the drug-resistance mechanisms. Our study provides an overall understanding of the distinct genomic landscape of ER+/PR-/HER2- breast cancer. Further investigations into the pathways activated with the loss of PR expression may provide clues for novel changes in the therapeutic regimens of ER+/PR-/HER2- breast cancer.
Title: Use of principal component analyses to select ER-balanced subset for gene centering in PAM50 subtyping

Praveen-Kumar Raj-Kumar1, Jianfang Liu1, Albert J Kovatich2, Leonid Kvecher1, Craig D Shriver3 and Hai Hu1, 1Chan Soon-Shiong Institute of Molecular Medicine at Windber, Windber, PA; 2Clinical Breast Care Project, Murtha Cancer Center, Uniformed Services University / Walter Reed National Military Medical Center, Bethesda, MD and 3Murtha Cancer Center, Uniformed Services University / Walter Reed National Military Medical Center, Bethesda, MD.

Body: Background: PAM (Prediction Analysis of Microarray) 50 is an established gene expression-based algorithm to classify breast tumors into basal-like, HER2-enriched, luminal A (LA), and luminal B (LB) subtypes. Clinical subtyping is mainly based on immunohistochemistry (IHC) assays of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (Her2) and Ki67 classifying tumors into triple-negative (ER-/PR-/Her2-), Her2+ (ER-/PR-/Her2+), LA (ER+/Her2-/Ki67-), LB1 (ER+/Her2-/Ki67+) and LB2 (ER+/Her2+). These two subtyping methods do not completely match even on comparable subtypes. Nevertheless, the ER-balanced subset for gene-centering in PAM50 subtyping was selected based on clinical status. Here we explored the possibility of using principal component analyses and iterative PAM50 call to refine the selection of an ER balance subset to improve consistency between these methods focusing on LB calls which is more aggressive than LA tumors.

Methods: Normalized gene expression data was obtained from TCGA research network for 712 primary tumors which had IHC status available for ER, PR and Her2. Since Ki67 status was not available LA and LB was discriminated for ER+ cases with Her2- and Her2+ respectively. In house RNA-Seq dataset had 118 primary tumors and were drawn from the Clinical Breast Care Project where breast cancer patients were consented using an IRB-approved protocol. Tumors were selected and processed by laser microdissection. RNA was extracted from tissues using the Illustra triplePrep kit (GE Healthcare). Paired-end mRNA sequencing was performed using the Illumina HiSeq platform. Sequenced reads were processed using PERL based pipeline utilizing PRINSEQ, GSNAP and HTSeq. Principal component analysis (PCA) was done using R. Wilcoxon rank sum test was used for statistical significance (p<0.05).

Results: In both datasets, the PCA map grouping of cases does not perfectly reflect the clinical subtypes. This motivated us to select ER balance subset based on the PC1 separation and IHC subtype. The resulting PAM50 subtypes on PCA map distinguished Basal and LA as two well separated components. Using all of Basal and equal number of LA cases for ER balance subset for PAM50 resulted in increased LB call and a better consistency with IHC LB calls. Among 712 cases in TCGA LB numbers increased from 142 in initial PAM50 call to 203 in ER balanced refined PAM50 call. We noticed that there was significantly higher (p-value = 4.414e-11) MKI67 expression for the 39 cases switch from LA to LB between PAM50 calls. Similar trend was observed in our in-house dataset where majority of the IHC-LB1 cases was called as LB in PAM50. The new method increased LB call from 22 to 27 which in-turn increased consistency between molecular and clinical subtypes from 73 to 79 out of the total of 118 cases.

Conclusion: We show that an iterative PAM50 call coupled with PCA for selection of ER balance set potentially enhanced the consistency of the LB calls with clinical subtyping and that the tumors switched from LA to LB have high MKI67 expression. The views expressed in this article are those of the author and do not reflect the official policy of the Department of Army/Navy/Air Force, the Department of Defense, or U.S. Government.
2017 San Antonio Breast Cancer Symposium

Publication Number: P2-07-01

Title: Integrative analyses of immunophenotypes and multi-omics profiles in breast cancers

Zhengyan Kan, Eric Powell, Sripad Ram, Keith Ching, Ying Ding, Pamela Vizcarra, Tim Nichols, James Hardwick, Soo-Hyeon Lee, Soo Youn Cho, Yoon-La Choi, Jong-Han Yu and Yeon Hee Park. 1Pfizer Oncology Research, San Diego, CA; 2Pfizer Korea, Seoul, Korea and 3Samsung Medical Center, Seoul, Korea.

Body: The advent of immuno-oncology (IO) therapies has made it an imperative to characterize intratumoral immune microenvironment in addition to oncogenic alterations through molecular profiling of the tumor. To elucidate the baseline profiles of tumor infiltrating leukocytes (TILs) in breast cancer (BC) in the context of molecular subtypes and oncogenic alterations, we performed whole-exome sequencing (WES) and RNA-Seq of an Asian BC cohort (SMC) consisting of 178 treatment naïve primary tumors. A subset of 120 tumors was further analyzed by H&E and IHC using a panel of 8 TIL markers (CD45, CD4, CD8, CD163, PD1, PD-L1, IDO1 and FOXP3). Using expression signatures representing distinct immune cell types, we classified an expression compendium of 2,781 tumor samples, including SMC and multiple cancers from TCGA, into three immune subtypes with high, medium and low levels of TILs. Basal and HER2 subtypes show higher levels of TILs than Luminal subtypes, consistent with observed clinical responses to checkpoint blockade in clinical trials. Moreover, Asian BCs were significantly enriched in TIL-high subtype (35.3%) compared to the primarily Caucasian TCGA BC cohort (20.2%) while 50.6% of the highly immunogenic Lung adenocarcinoma was TIL-high. We then applied machine learning methods to detect and quantify TILs from H&E images of 120 SMC and 349 TCGA BC tumors. The expression signature analysis results were concordant with independently derived histology based TIL data. Taken together, our findings suggest that IO therapies may be more effective in HR negative BC subtypes and Asian BCs.

Leukocyte exclusion (LE), an immunophenotype where TILs concentrate at the tumor periphery, has been linked to worse prognosis and resistance to IO therapies. Visual assessment of whole tumor IHC images identified LE patterns in 25% of SMC cases. We observed differential distribution of LE by molecular subtype and evidence for selective exclusion of immune cell subsets. Covariate analyses with clinical and molecular data while controlling for subtype as a confounder identified significant associations with tumor proliferation index, percent tumor purity and TP53 mutations. LE is also significantly associated with expression signatures of chemokine signaling, macrophages, angiogenesis and hypoxia, indicating that marked distinctions exist in both tumor intrinsic and microenvironment characteristics between TIL excluded and TIL infiltrated tumors. To validate these findings, we independently identified LE for 200 cases of TCGA BCs based on patterns of TILs extracted from H&E images and saw significant concordance of covariate relationships identified between TCGA and SMC. Our study provided a rare comprehensive resource for studying tumor associated immunity in breast cancers by generating the integrated multi-omics and IO profiles for a large cohort of primary tumors. Comparative analyses revealed that TIL activities are highly variable across different intrinsic subtypes and geographic origins of BC, with potential implications for IO therapeutic application. Correlative analyses of immunophenotypes with molecular data further yielded insights into LE’s role in immune escape and identified hallmark signatures for LE indicative of causal molecular mechanisms.
Title: Uncovering TNBC molecular heterogeneity by applying integrative “omics” analyses with potential clinical implications

Yacine Bareche¹, David Venet¹, Françoise Rothe¹ and Christos Sotiriou¹. ¹Breast Cancer Translational Research Laboratory, Jules Bordet Institute, Université Libre de Bruxelles, Brussels, Belgium.

Body: Introduction: Triple-negative breast cancer (TNBC), representing about 10-20% of all breast cancers (BC), is a heterogeneous disease characterized by a clinically aggressive course, higher relapse rates and worse overall survival as compared to other BC subtypes. Recent efforts of genome-wide gene expression profiling have improved our understanding of the biological diversity of TNBCs reporting at least 6 different molecular subtypes namely Basal-like 1 (BL1), basal-like 2 (BL2), immunomodulatory (IM), mesenchymal (M), mesenchymal stem-like (MSL) and luminal androgen receptor (LAR). Little is known regarding the potential driving molecular events characterizing each subtype, their difference in survival and response to therapy. Moreover, limited progress has been made so far in the development of effective therapeutic strategies in TNBC partly due to the lack of obvious genetic targets. Further insight into the underlying genomic alterations is therefore needed.

Aims: Here, we aimed to study the genomic aberrations that drive each of the TNBC molecular subtypes as defined by Lehmann et al. by applying an integrative analysis combining somatic mutation, copy number aberrations and gene expression profiles of 272 TNBC derived from METABRIC consortium.

Methods: In silico analyses were performed using microarray, copy number aberrations profiles (CNA) and mutation profiles data retrieved from 272 TNBC patients from the METABRIC consortium. Somatic mutation profiles were derived from targeted sequencing of 173 cancer genes. CNAs at the gene level were computed using GISTIC 2.0.22. The relationship between TNBC molecular subtypes and genomic aberrations were analysed using Fisher test. Survival analyses were performed using Cox proportional hazard models adjust for standard clinical and pathologic variables.

Results: Using a multivariate model, IM and M subtypes were significantly associated with good (HR=0.53; FDR=0.01) and poor (HR=1.8; FDR=0.01) prognosis respectively. BL1 subtype was found to be the most genomically instable subtype with high frequency of TP53 mutations (93%) and copy-number deletions in genes involved in DNA repair mechanisms (BRCA2, MDM2, RB1 and TP53) and MYC overexpression. BL2 subtype was enriched with TP53 (95%) and PIK3CA mutations (52%), whereas LAR subtype was characterized by high frequency of PI3KCA mutations (44%). Of interest, for these two subtypes, mutations in genes in the PI3K signalling pathway were mutually exclusive. IM subtype was enriched in NCOR2 mutations (13%), a co-repressor of the Notch pathway, associated with lower gene expression (p=0.035). M and MSL subtypes were significantly associated with higher signature scores for angiogenesis. In addition, M subtype was associated with higher frequency of NOTCH1 mutations (M p=2.4E-2).

Conclusions: This integrative analysis combining somatic mutation, copy number and gene expression profiles shed new light on TNBC molecular heterogeneity, highlighting TNBC subtype-specific genomic aberrations which could potentially open new avenues for the development of effective targeted therapies in each TNBC molecular subtype such as DNA repair, Notch and angiogenesis.
Title: Systematic characterization of kinase inhibitors reveals heterogeneity in responses by class and cell line

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Body: Several publications have addressed concerns surrounding drug response screens by pointing out sources of variability and by presenting recommendations for better experimental methods and more robust analytical approaches. In the presented profiling effort, we integrated the latest advances in drug response measurement and focused on data diversity and quality rather than on breadth. We selected 32 breast cancer cell lines with a strong bias towards triple negative lines as well as 4 cell lines established from relevant patient-derived xenografts. We evaluated a panel of clinically relevant kinase inhibitors using a microscopy-based dose response assay to measure drug potency, and to quantify drug efficacy in terms of growth inhibition (GR metrics) and cell death. The use of the GR metrics to quantify drug sensitivity enabled us to identify and study differences between cytostatic and cytotoxic responses. This systematic dose response dataset is complemented by measurements of baseline transcript expression levels by mRNAseq, quantification of absolute abundance of ~12,000 proteins, and relative phosphoprotein levels by shotgun mass spectrometry across all cell lines. Additionally, the baseline activity of transcription factors and kinases were inferred from the mRNA (using VIPER) and phosphoprotein (using kinase enrichment analysis) data, respectively. The complementarity of these multi-omics data has allowed us to address questions about the landscape of breast cancer cell lines such as: Where do the patient-derived lines lay relative to the conventional cell lines? How consistent are the landscapes defined by each dataset? How does integration across datasets provide mechanistic insight into signaling pathways that are active in each cancer subtypes? The measured and inferred baseline data were used to build predictors of the observed drug responses with the goal of identifying the biological processes responsible for the differences in sensitivity across drugs and cell lines. Overall the dataset that has been collected is a valuable resource for understanding drug response in triple negative breast cancer, and the molecular mechanisms that influence it.
Title: Molecular regulators of resistance and relapse in chemorefractory triple-negative breast cancers

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Body: Triple-Negative Breast Cancer (TNBC) accounts for approximately one-fifth of breast cancer incidence but disproportionately high mortality. Two-thirds of early-stage TNBCs are resistant to pre-surgical chemotherapy and highly prone to relapse within 3 years. Moreover, no advanced therapies are indicated for patients with these cancers. We have embarked on a comprehensive genomic analysis of chemoresistant TNBC to gain an in-depth understanding of molecular entities driving chemoresistance and relapse. By collecting somatic mutation and copy number, RNA-sequencing, and outcome data in the context of a phase II post-neoadjuvant clinical trial, we have uncovered several molecular mechanisms behind these aggressive cancers. Through the analysis of matched pairs sampled before and after chemotherapy, we have discovered multiple means by which tumors are able to overcome the effects of chemotherapy including clonal evolution of high-level oncogene amplification, repression of the in situ immune system, and upregulation of the stem cell-related MEK-ERK and JAK-STAT pathways. Investigation into factors related to prognosis revealed important correlations between relapse and immune and JAK-STAT signaling. Finally, using a novel method of demarcating loss-of-function of p53, which we have termed graduated inactivation, we discovered additional associations between p53 loss and relapse, mortality, and MYC signalling.
Title: Genomic lesions and PD-1/PD-L1 expression in resected triple negative breast cancers

Body: Introduction: Striking clinical responses to immunotherapies in subsets of patients with a variety of solid tumors have prompted the search for predictive biomarkers. Recent studies have proposed that gene specific and genome wide mutation and copy number signatures in tumor cells may be predictive of responses to immune checkpoint blockade. Notably high levels of PD-L1 on tumor cells, a context associated with an adaptive immune response, has been linked to specific oncogenic driver lesions including loss of PTEN, activating KRAS mutation, and MYC amplification, and to the total burden of copy number variants (CNVs) in aneuploid tumors. Triple negative breast cancers (TNBCs) typically have multiple driver mutations and high levels of CNVs in their genomes. Thus there is significant interest in exploiting genomic data for the development of prognostic immunotherapy biomarkers for patients with TNBC.

Study Design: We interrogated 62 well annotated surgical resections from patients with TNBC and assessed the associations of genomic lesions with expression of PD-1 and PD-L1 in tumor and non-tumor cells in each sample. We applied a systematic approach to rigorously interrogate the genomes of each TNBC sample. Tumor ploidy was initially measured with DNA content flow cytometry followed by sorting the nuclei of distinct diploid, tetraploid, and aneuploid cell populations from each TNBC. The next level of analysis measured genome wide copy number variants (CNV) with oligonucleotide arrays designed for CNV detection using purified (>95%) tumor populations. This enabled the discrimination and mapping of CNVs including single copy losses and gains, focal amplifications, and homozygous deletions across each TNBC genome. Finally we generated whole exome data in a subset of samples to increase the resolution within loci of interest and to incorporate mutations of individual genes into our genomic signatures. This combined approach provides high resolution measures of TNBC genomes from ploidy, whole chromosome and chromosome arm level CNVs, focal amplicons, breakpoints and homozygous deletions across each TNBC genome. Finally we generated whole exome data in a subset of samples to increase the resolution within loci of interest and to incorporate mutations of individual genes into our genomic signatures. This combined approach provides high resolution measures of TNBC genomes from ploidy, whole chromosome and chromosome arm level CNVs, focal amplicons, breakpoints and homozygous deletions, to the level of gene specific indels and mutations. In parallel whole tissue samples were screened by IHC for PD-1 and PD-L1 using validated antibodies and established scoring methods for staining of tumor and non-tumor cells.

Results and Conclusions: High levels of PD-1 and PD-L1 were detected in 5/60 (8.3%) and 16/60 (26.7%) evaluable cases with staining of PD-1 exclusively on non tumor cells, while PD-L1 was primarily on tumor cells; 15/16 (93.8%) cases. These data were then used to test the associations of individual recurring genomic lesions, and the extent and nature of chromosome aberrations on the expression patterns of PD-1 and PD-L1. Homozygous deletion of PTEN or activating mutation in PIK3CA did not correlate with increased expression of either immune checkpoint regulator in TNBC cells. Furthermore tumors with highly aberrant aneuploid genomes and distinct oncogenic drivers frequently express relatively low levels of both proteins suggesting an intrinsic escape from immunosurveillance.
Title: Multi-omics characterization of claudin-low breast tumors

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Body: Breast tumors display highly heterogeneous characteristics both at transcriptional level and in term of genomic landscape. We recently reported that the differentiation status of the cell-of-origin influences the genetic route toward tumorigenesis¹. Indeed, mammary stem cells exhibit the intrinsic property to tolerate the oxidative stress inherent to the oncogenic transformation through the expression of a preemptive program driven by ZEB1, an epithelial-to-mesenchymal transition (EMT)-related transcription factor, and MSRB3, a methionine sulfoxide reductase. Thereby, ZEB1-expressing tumors exhibit low level of DNA damage associated with few genomic alterations and low level of TP53 mutations. Importantly, these tumors share several transcriptional characteristics and features with normal stem cells, and with Claudin-Low (CL) molecular subtype of breast cancers. In the present work, we attempt to decipher molecular traits of CL breast tumors through multi-omics analyses of publicly available databases. This global approach allows us to highlight various CL breast tumors features as clinical attributes, gene expression, copy number alterations (CNA), somatic mutations or drug responses by differential analyses of breast tumors and cancer cell lines. Preliminary results indicate that, independently of tumor purity, CL breast tumors are mostly diploids, present a paucity of genomic rearrangements and a lower mutation rate compared to other breast tumors. They mainly, but not exclusively, show basal features and belong to the integrative cluster 4 (CNA-devoid) described by Christina Curtis and colleagues². Concerning gene expression, CL breast tumors exhibit a frequent activation of RAS signaling pathway, an observation consistent with their sensitivity to MAPK inhibitors. Other analyses are currently ongoing and aim to better understand the biology of CL breast tumors in order to improve both the diagnosis and the therapeutic strategy used.

Title: Exploring novel therapeutic target molecules for metaplastic breast carcinoma using comprehensive genome-wide gene expression analyses

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Body: Breast cancer subtypes have been classified based on the gene expression profiles, and the specifically effective treatments for each subtype have been developed and applied in clinical situations. However, there is no effective treatment for metaplastic breast carcinomas, which are pathological tissue types with poor prognosis. Metaplastic breast carcinomas are almost resistant to existing chemotherapy because it has not been identified the specific therapeutic target molecules. Recently, Lehmann et al. reported that breast cancers with chondroid metaplasia are categorized in Mesenchymal (M) type, which is one subtype out of six triple negative breast cancer (TNBC) subtypes based on drug susceptibility. From the knowledge on this report, we hypothesized that integrative analyses using the gene expression profile data sets obtained from various types of breast cancers with or without cartilage formation may enable us to identify the therapeutic target genes for metaplastic breast carcinomas, especially for breast cancers with chondroid metaplasia.

Bioinformatics analyses were performed using the gene expression profile data sets of TNBC, which are registered in Gene Expression Omnibus (GEO). When compared with metaplastic breast carcinomas (28 data sets), 200 genes were specifically fluctuated over 2-fold in breast cancer with chondroid metaplasia (8 data sets). On the other hand, M type (11 data sets) specific 578 genes were identified over 56 TNBC data sets. In addition, 57 genes were overlapped between chondoroid metaplastic 200 genes and M type 578 genes. Therefore, these 57 overlapped genes might be considered as possible poor prognostic factors in breast cancer with chondroid metaplasia. As a result of Gene Ontology analysis on these 57 genes, skeletal system development and extracellular matrix structural constituent were significantly enriched in Biological process and Molecular function, respectively. Also, \textit{SCRG1}, \textit{SOX8} and \textit{SOX10}, which are genes related to cartilage differentiation, were included.

Next, we focused 14 genes because these genes could serve as therapeutic targets. Then, we validated the expression levels of these 14 genes using tissues obtained from surgically resected human breast cancer with chondroid metaplasia dividing into three parts, the invasive ductal carcinoma, the chondroid metaplasia and normal mammary gland. As a result, \textit{SCRG1}, \textit{CD86}, \textit{HCLS1}, \textit{EPSTI1}, \textit{LYZ}, and \textit{SLA} were significantly increased in chondroid metaplasia part, suggesting that these validated genes were identified as possible therapeutic targets for breast cancer with chondroid metaplasia. Further study will help us to investigate a novel and effective therapeutic strategy for metaplastic breast carcinomas.
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Title: Prognostic relevance of claudins 4 and 7 in invasive breast carcinoma (NOS) subtypes: A large tissue microarray study

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Body: Molecular phenotyping has improved the understanding of a wide range of breast cancer disease. Recently, a new molecular subtype denominated “claudin-low” (CL) was described in breast cancer and correlated to worse prognosis and to CD44+/24- stem cell profile. Among 19 known claudin proteins, isotypes 4 (CL4) and 7 (CL7) are the most common in the breast biology. 

Objective: To verify differences in CL4 and CL7 immunoexpression between Luminal A, HER-2, and triple negative breast cancer phenotypes; and their association to CD44/24 status and tumor prognosis. 

Design: Estrogen and progesterone receptor status (ER/PR), HER-2, CL4 and CL7 expression and CD44/24 profiles were evaluated in 803 invasive ductal breast carcinomas arranged into four tissue microarrays (TMA) and results were correlated with prognosis and important clinical data.

Results: 503 (62.6%) cases were positive for CL4 and 369 (46.0%) cases for CL7. The majority (199/283, 70.3%) of CL4 negative cases were clustered in the luminal A subtype whereas 63 (22.3%) showed triple negative profile and the remaining 21 cases (7.4%) exhibited positive HER-2 expression (p<0.001). Claudin 7 negative samples (44.2%) tended to follow the same pattern. CL4 positive expression was significantly associated to HER-2 expression, presence of lymph nodes and increased tumor grades and inversely correlated to ER and PR expression. However, there was no association between CL7 expression and any of these features. Both CL4 and CL7 did not show correlation to the stem cell markers (CD44+/CD24+) or worse prognosis (survival and disease-free interval).

Conclusion: Claudins 4 and 7 immunoexpression did not provide additional prognostic information within breast cancer subtypes.
Title: Integrative analysis of miRNA and mRNA expression in metastatic versus non-metastatic triple negative breast cancer

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Body: Background: Triple Negative Breast Cancer (TNBC) is a subset of breast cancer that is difficult to treat clinically and is characterized by being estrogen receptor (ER) negative, progesterone receptor (PR) negative, and does not overexpress human epidermal growth factor receptor 2 (HER2). Patients with TNBC tend to have a worse prognosis than other breast cancer subtypes.

Methods: We obtained fifteen TNBC sample FFPE tissue blocks with corresponding plasma samples. All samples were from primary tumors; seven samples having metastasized, four samples that had not metastasized and four samples with unknown metastatic status. The total RNA was isolated from FFPE blocks using the RecoverAll Total Nucleic Acid Isolation Protocol. miRNA from plasma was isolated using Ambion's mirVANA kit. The plasma and tissue miRNAs were evaluated using the QuantStudio qPCR platform, capturing ~750 miRNAs. The mRNA was processed using the TruSeq RNA Access kit and sequenced on the Illumina NextSeq platform. Analysis of the miRNA and mRNA individually was performed using limma and DESeq2 packages, respectively. Gene enrichment analysis of the mRNA expression was done using the GAGE package on KEGG pathways while the integrative analysis was done with sparse Canonical Correlation Analysis (sCCA) using the PMA package.

Results: Analysis of plasma miRNA had four miRNAs with a significant difference in raw p-value (p < 0.05) between metastatic and non-metastatic TNBC; miRNA 708, 483-3p, 518f, and 766; in the tissue there were fifteen miRNA with p < 0.05, with one miR-872 still having significance after adjusting for multiple testing. mRNA had 33 genes being significant after multiple testing correction with several immune KEGG pathways being downregulated in metastatic samples (adjusted p < 0.05). The integrative analysis revealed five microRNA (miR-216, miR-127, miR-370, miR-382, and miR-487b) and 312 gene modules enriched in integrin (Fisher p < 10^-5) and extracellular matrix (Fisher p < 10^-6) signaling.

Conclusions: One of the circulating plasma miRNAs, miR483-3p, has been found to promote tumorigenesis, while miR581f and miR766 have not been reported in cancer to date. Further investigation into these miRNA could provide a feasible biomarker. The downregulation of immune pathways observed within the metastatic TNBC subjects implies immune evasion is of particular importance for metastasis and a targeted immunotherapy may be a viable treatment option. The integrative analysis of the miRNA and mRNA showed an enrichment in pathways previously linked to increased proliferation and chemoresistance, with an increased signal compared to either miRNA or mRNA alone.
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Title: Loss of function of Brca1 or Gata3 induces basal-like breast cancer

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Body: BACKGROUND:
Breast cancer is mainly divided into estrogen receptor (ER)-positive luminal and ER-negative basal-like tumors. Luminal-type tumors are associated with better survival and respond to hormone therapies whereas basal-like tumors are more aggressive and associated with a poor prognosis. Mammary epithelia are mainly composed of luminal and basal cells that are maintained by luminal and basal progenitors, respectively. The maintenance of luminal cell fate is orchestrated by networks of transcription factors, including BRCA1 and GATA3. Functional loss of BRCA1 by germline or somatic mutation or by promoter methylation is associated with more than one third of basal-like breast cancers. GATA3 expression is reduced in basal-like breast cancers and cancers that metastasize. Overexpression of GATA3 in cancer cells inhibits tumor formation. Deletion of Brca1 or Gata3 in mice results in early lethality or growth defects. How BRCA1 and GATA3 suppress breast cancer remains elusive.

METHODS:
We generated mice lacking Brca1 or Gata3 in mammary epithelia. Due to the proliferative defects and induction of p18Ink4c (p18), an inhibitor of CDK4/6, in mammary epithelial cells of these mice, we then generated mice lacking Brca1 or Gata3 in p18 deficient mammary epithelia. We determined spontaneous mammary tumor development in mutant mice and the mechanisms underlying the role of Brca1 and Gata3 in suppressing tumorigenesis and progression.

RESULTS:
Depletion of Brca1 or Gata3 led to growth defects of mammary epithelial cells, which was rescued by loss of p18. Depletion of Brca1 or Gata3 in a p18 null background induced heterogeneous mammary tumors with less luminal and more basal-like features and accelerated metastasis. Deletion of Brca1 eliminated Gata3 expression in human and mouse mammary tissues and cells. How Brca1 interacts with Gata3 to control mammary tumor development and progression is currently under investigation.

CONCLUSION: Our results suggest that loss of function of either Brca1 or Gata3 induces basal-like mammary tumors in p18 deficient background.
Title: Novel tumor suppressor regulating the PI3K/AKT pathway in breast cancer

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Body: Breast cancer affects approximately 1 in 8 women over the course of their lifetime. Of the many genes and pathways deregulated by this heterogeneous disease, alterations of members in the PI3K/AKT pathway are present in approximately 20% of all cases, making it one of the more frequently mutated pathways in breast cancer. The PI3K/AKT pathway is well known to be involved in essential cellular processes necessary for cancer development and progression, including altered proliferation, metabolism, and cell survival. Previous studies have made it well known that the activation of AKT is regulated through its phosphorylation, but recent studies have also shown that AKT can also be regulated via ubiquitination, leading to AKT hyperactivation. However, the mechanism and players involved in this process are not well understood. Better understanding of these pathways could inform on better potential therapeutics to target this disease.

Recent work in our lab has identified GPS2, a member of the NCoR/SMRT complex, as a regulator of the insulin-signaling pathway through the inhibition of AKT ubiquitination by Ubc13. As many human cancers frequently have an increase in activated AKT, here we explore the potential of GPS2 as a tumor suppressor in the context of breast cancer and hope to elucidate the mechanism by which AKT ubiquitination regulates its activation.
An essential role of GRB7 in promoting the growth of therapy resistant HER-2 positive human breast cancer cells in culture and animal models

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**Body:** Background - GRB7 gene encodes a multi-domain signal transduction molecule and is part of the core of the HER-2 amplicon. GRB7 is commonly co-amplified and over-expressed with HER-2 in human breast cancer. Earlier studies found a functional role of GRB7 in breast cancer. The role of GRB7 in HER-2 positive human breast cancer resistant to HER-2 targeted therapy remains unexplored however.

**Materials and Methods** - HCC-1954, 21MT1 and JimT1 are human HER-2 positive breast cancer cell lines that are resistant to trastuzumab and lapatinib treatment. Transient knock down of GRB7 protein expression was achieved with siRNA transfection and stable knock down with lentiviral vector mediated shRNA over-expression. Cell lines transfected with non-targeting siRNA or shRNA serve as negative controls. Knock down of GRB7 protein expression is verified by Western blotting. The growth of human breast cancer cell lines after GRB7 knock down in vitro is measured with the CellTiter Glo assay as well as the Incucyte live cell imaging. Activation status of specific signaling pathways was examined with phospho-specific antibody by immune-blotting and immune-precipitation. To assess the growth promoting function of GRB7 in human breast cancer cell lines in vivo, polyclonal HCC-1954, 21MT1 and JimT1 cells, with GRB7 knock down or their corresponding negative control, were orthotopically injected into the mammary fat pads of female immune-deficient NSG mice. The growth rates of these tumors, measured serially with caliper, and final tumor weights were compared between GRB7 knock down and the negative control. The proliferation rate and apoptosis of these tumors were studied with ki-67 staining and Tunel assay. The effects of GRB7 knock down on signaling were investigated with a proteome profiler receptor tyrosine kinase kit (R&D). The role of signaling molecules differentially activated in the growth of breast cancer cells by GRB7 knock down was examined utilizing siRNA mediated knock down, and antibody and small molecule inhibitors.

**Results** - GRB7 knock down decreased the growth of HCC-1954, 21MT1 and JimT1 cells in vitro and the growth of tumor xenograft these cells formed in animal models. When assayed by ki67 staining and Tunel assay, the mechanism of reduced tumor xenograft growth appeared to be distinct. Reduced proliferation and increased apoptosis were seen in 21MT1 cells, while reduced proliferation was seen in HCC-1954 cells and increased apoptosis in JimT1 cells. Protein profiling found that tyrosine phosphorylation of candidate signaling molecules was reduced with GRB7 knock down in JimT1 cells. Immuno-blotting and immuno-precipitation experiments were performed to evaluate these effects in other cell lines. The effect of targeting these molecules in breast cancer cell growth by siRNA and inhibitors is being examined.

**Discussion** - GRB7 has essential growth promoting function in therapy resistant HER-2 positive human breast cancer cells. GRB7 knock down has pleiotropic effects on signaling in various cellular contexts. The potential of targeting GRB7 signaling in treating therapy resistant HER-2 positive breast cancer merits further study.
body:

**Background:** Triple negative breast cancer (TNBC) presents a very aggressive behavior with a high rate of metastasis. Overexpression of HMGA1 has been reported in TNBC and has been associated with the induction of the Epithelial-Mesenchymal transition (EMT) and metastasis. Therefore, HMGA1 is considered a master regulator of tumor progression in TNBC. The objective of this work was to know which genes are directly or indirectly regulated by HMGA1 to better understand their participation in EMT and their role in aggressive TNBC.

**Methods:** We performed the silencing of the HMGA1 gene using siRNA Silencer® Select Pre-designed (s6667 HMGA1, 4390849 GAPDH, 4390843 Negative control, all from Thermo Fisher, MA, USA) in two TNBC cell lines, HCC-1395 and MDA-MB-231, and we observed the effect of this gene inhibition by microarray global expression analysis using GeneChip Human Genome U133 Plus 2.0 (Affymetrix, CA, USA), comparing the conditions of inhibition versus their own control without inhibition. After the microarray data mining, results for the HMGA1 and PRRX1 genes were validated by qPCR using the Prime Time® Primers for HMGA1 (Hs.PT.58.38699366), PRRX1 (Hs.PT.58.2820749), and GAPDH as endogenous gene (Hs.PT.39a.22214836) with SybrGreen reagent (Roche, Basel, Switzerland). The level of expression of the HMGA1 and PRRX1 proteins was analyzed by Western blot in nuclear protein extracts of each cell line before and after gene silencing. Finally, we performed an in silico analysis using the “Gene 2 promoter” tool in the Genomatix platform to search the promoters and binding proteins of the PRRX1 gene.

**Results:** The silencing of HMGA1 in a non-metastatic TNBC cell line, HCC-1395, showed deregulation of genes associated with cell proliferation and angiogenesis. Meanwhile the silencing in a TNBC-metastatic cell line, MDA-MB-231, resulted in the deregulation of genes involved in the formation and organization of the cytoskeleton, including the overexpression of PRRX1. Validation of the expression changes of HMGA1 and PRRX1 by qPCR and Western blot was performed and HMGA1 was confirmed to negatively regulate the PRRX1 gene expression. Through in silico studies, we identified several binding sites of HMGA1 to the PRRX1 promoter.

**Conclusions:** The subexpression of PRRX1 is necessary for EMT to occur. In this study, we present the interesting finding that HMGA1 regulates the subexpression of PRRX1, as supported by the experiments of transcriptional and translational expression presented in this work. To our knowledge, this is the first report describing a regulatory role of HMGA1 on PRRX1, which could explain the metastatic capacity of cancers that overexpress HMGA1.

**Key words:** Epithelial-Mesenchymal Transition, Triple Negative Breast Cancer, Mesenchymal-Epithelial Transition, Metastasis.
Title: FOXK2 aberration in breast cancer

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Body: Background: Activation of oncogenes through DNA amplification/overexpression plays important roles in cancer initiation and progression. The chromosome 17 is a frequent site of cancer-associated genetic anomalies. This cytogenetic anomaly is strongly associated with poor prognosis and is a significant predictor of relapse in breast cancer. Previous studies of breast cancer have revealed the amplification of several genomic regions on 17q. These amplifications are typically discontinuous and complex in structure, suggesting that multiple oncogenes in this chromosomal segment may be co-selected during breast carcinogenesis. By integrative analysis of public genomic datasets of breast cancers from The Cancer Genome Atlas (TCGA), we have found that FOXK2 displayed frequent genomic amplifications and correlated gene expression changes in breast cancer. FOXK2 gene is located in 17q25 and encodes a transcriptional factor with a fork head DNA binding domain, but has not yet been reported to be associated with cancer-causal genetic aberrations. Gene amplification in the 17q chromosomal region is observed frequently in breast cancers. We hypothesize that FOXK2 is an important oncogene in breast cancer and it might be a novel therapeutic target and biomarker for breast cancer.

Methods: The status of FOXK2 located at the Chromosome 17q25 was explored by mining the breast cancer TCGA datasets including 910 tumor cases and 981 normal controls. To determine whether FOXK2 amplification/overexpression is required for breast cancer cell proliferation, we assessed the effect of FOXK2 stable knockdown on proliferation and anchorage-independent growth in four cell lines with high FOXK2 expression status (MDA-MB-231, MCF-7, HCC1954 and MDA-MB-361) using lentivirus mediated shRNAs. The oncogenic activity of FOXK2 was assessed by colony formation assay. The potential interacting molecules/pathways were explored.

Results: Frequent genomic amplifications of FOXK2 were detected in breast cancers compared to normal controls in all subtypes of breast cancers classified by PAM50 by integrative analysis of public genomic datasets and its overexpression was associated with poor overall survival of breast cancer patients. FOXK2 knockdown in several breast cancer cell lines inhibited breast cancer cell proliferation and anchorage-independent growth. More importantly, overexpression of FOXK2 and oncogene RAS induced MCF10A cell colony formation, indicating that FOXK2 is an oncogene in breast cancer. Several pathways, including regulation of cell proliferation, regulation of cell division, cell adhesion and regulation of cell metabolism, were regulated by FOXK2 in breast cancer cells.

Conclusion: Our data provide compelling evidence that FOXK2 is an oncogene in breast tumorigenesis, and it might be a novel therapeutic target and a biomarker predicting poor outcome. We are assessing the incidence of FOXK2 amplification in human breast cancer specimens using immunohistochemistry and FISH on TMA tissue blocks to determine the prognostic value of FOXK2 amplification for overall prognosis and treatment resistance. We are also validating the molecular mechanism of FOXK2 in breast cancer malignancy according to the identified potential interacting molecules/pathways by analyzing the RNASeq data of a breast cancer cell line with FOXK2 knockdown.
Title: T-cell receptor beta chain variable region (TRBV) expression patterns predict response to combined trastuzumab/lapatinib treatment in the NeoALTTO/BIG-1-06 trial

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Body: Background: Dual anti-HER2 blockade resulted in increased pathologic complete response rate (pCR) in the 3 arm NeoALTTO trial. High immune gene expression and the absence of PIK3CA pathway mutations are predictive of pCR in all treatment arms but no markers were identified that could predict which patients require dual HER2 targeted therapy. The goal of this analysis was to examine if TRBV expression could add to the predictive function of previously identified immune markers. Patients and Methods: We analyzed RNA and Whole Exome sequencing data from 245 cancers (54% of all patients) included in the trial. The TRBV reference sequences were obtained from the International ImMunoGeneTics information system. Reads were aligned using a custom BLAST mapping pipeline and normalized by the total number of aligned reads in each sample. We calculated 3 T cell receptor metrics for each tumor including (i) total TRBV chain expression level, (ii) Shannon entropy of the normalized unique TRBV-expression frequencies which reflect TCR diversity and (iii) we also used non-negative matrix factorization (NMF) to define TRBV co-expression metagenes (TRBVMG). We evaluated correlation between these metrics and immune and proliferation gene expression signatures and genomic features of the cancer including clonal heterogeneity and mutation load. We assessed association between TRBV and pCR using multivariate logistic regression. Results: 65 distinct TRBV variants showed heterogeneous expression levels across cancers with strong co-expression patterns. Total TRBV expression correlated strongly with immune metagene expression (Spearman's ρ=0.93, P<0.001), but entropy had a weaker, inverse correlation with immune metagene expression (Spearman's ρ=−0.40, P<0.001). Associations between TRBV metrics and mutation load and clonal heterogeneity were weak. pCR correlated with higher total TRBV expression (Spearman's ρ=0.17, P<0.05). Correlation between entropy and pCR was non-significant (odds ratio (OR) for regressing entropy with pCR was <1). NMF identified 4 distinct TRBVMGs that showed substantial expression variation within immune cell rich cancers. ER-status, proliferation and immune-gene expression adjusted logistic regression analysis including a treatment-arm interaction term revealed that TRBVMG-2, characterized by high expression of TRBV4.3, TRBV6.3 and TRBV7.2 variants, was associated with higher pCR rate in patients treated with trastuzumab plus lapatinib (Interaction OR=3.23 adjusted P=0.03). In immune-rich cancers, TRBVMG-2 expression above the median was associated with higher pCR rate in the dual HER2 targeted treatment arm compared to the other arms (68% vs 21%, Fisher exact test P<0.001). Patients with immune cell rich cancers but TRBVMG-2 expression below the median had similar pCR rates in all arms (42% monotherapy vs. 28% dual therapy, P=0.46). Conclusions: TRBV expression pattern can provide predictive information beyond known immune gene expression signatures. High expression of TRBV4.3, TRBV6.3 and TRBV7.2 variants is associated with higher pCR rate with dual HER2 targeted and paclitaxel neoadjuvant therapy.
Title: Blinded molecular subtyping analysis from RNA-Seq of FFPE samples in the GeparQuinto trial reveals predictive value of VEGFA metagene for bevacizumab treatment

Body: Background:
RNA-Seq from total RNA in FFPE tissue can be more challenging due to limited capture of partially degraded RNA. Exome-capture based RNA-Seq may circumvent such problems and allow reproducible complete molecular characterization of low-quality RNA from small clinical samples.

Methods:
HER2 negative patients within the GeparQuinto trial were treated with neoadjuvant anthracycline-taxane-based chemotherapy +/- bevacizumab. Patients with bevacizumab therapy had a significantly higher pCR rate, especially within the triple negative subgroup. We performed exome-capture RNA-Seq on 5µm FFPE sections from pre-therapeutic cores of 400 HER2 negative samples from this trial. In a prospectively planned, blinded study we correlated molecular subtypes and metagenes for proliferation, stroma, MHC2, and VEGFA with clinical and histopathological data. Molecular subtypes were defined using the AIMS methods. Metagenes were calculated as mean values corresponding to previously described gene clusters after platform transfer (Rody et al. 2011 PMID 21978456, Hu et al. 2009 PMID 19291283) and then z-transformed.

Results:
296 samples with RNA-Seq data were classified as either of high (n=226) or of limited quality (n=70). For 22 samples RNA yield was insufficient and 82 did not pass initial QC. 121 (41%), 63 (21%), 34 (11.5%), 46 (15.5%), and 32 (11%) samples were defined as basal-like, HER2-enriched, luminal A, luminal B, and normal-like, respectively. Subtyping was robust with regard to gene filtering, normalization, and sample quality. ER and PR status from local IHC strongly correlated with gene expression (overall correctness 84% and 80% for ER, and 85% and 74% for PR, in samples with high and limited quality, respectively) and luminal subtypes (95% ER positive). Proliferation metagene correlated with histological grade (median -0.73, -0.39, and 0.53 in G1, G2, and G3, respectively; P<0.001) and MHC2 metagene correlated strongly with TIL counts (Rho=0.53, P<0.001). Among the high quality samples response rates (49.3% pCR overall) differed significantly by subtype, with higher pCR rates in basal-like (68.9%) and HER2-enriched (45.5%) than in luminal B (35.7%), luminal A (17.9%), and normal-like (20.0%). MHC2- (OR 1.60, 95%CI 1.21-2.12, P=0.001), proliferation- (OR 2.88, 95%CI 2.00-4.16, P<0.001), and VEGFA-metagenes (OR 1.92, 95%CI 1.41-2.60, P<0.001) were significant predictors for pCR. In a multivariate logistic regression (adjusted for bevacizumab treatment and hormone receptor status) both VEGFA metagene (OR 2.59, 95%CI 1.40-4.77, P=0.002) and the interaction between the VEGFA-metagene and bevacizumab treatment arm (P=0.023) significantly predicted pCR.

Conclusions:
Exome-capture RNA-Seq allows robust genomic characterization of clinical samples with limited FFPE material from core biopsies, and molecular subtypes and immune metagenes are predictive for pCR. The VEGFA metagene is a specific predictor for response to neoadjuvant bevacizumab treatment.
Title: Identifying clinically relevant subgroups of women with HER2-positive breast cancer: An analysis of Neo-ALTTO using the 41-gene TRAR score

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Body: BACKGROUND: As a neoadjuvant regimen for HER2-positive early breast cancer (BC), the use of two HER2-directed agents is more effective in producing pathological complete (pCR) responses than trastuzumab alone. Nevertheless, highly effective dual anti-HER2 combination may be unnecessary in patients who already benefit from a single agent. We previously reported that our 41-gene TRAR score is an accurate predictor of response to trastuzumab, with low scores being predictive of response to trastuzumab and favorable prognosis (Triulzi T. et al., 2015).

PATIENTS AND METHODS: Tissue specimens from HER2-positive BC patients of Neo-ALTTO trial who received neoadjuvant trastuzumab and/or lapatinib plus paclitaxel were included in this study. Analysing RNA from fresh tissue using the 41-gene signature test, the area under the ROC curve (AUC) and the corresponding 95% confidence interval was computed to evaluate the predictive ability of TRAR score with respect to pCR, the primary endpoint of Neo-ALTTO. The prognostic role of TRAR score was investigated using a Cox regression model in univariate fashion. The patterns of Event Free Survival (EFS) according to the dichotomized TRAR score were estimated using the Kaplan–Meier method.

RESULTS: The TRAR score was assessed for 226 of the 455 (49.7%) patients enrolled in the Neo-ALTTO study: 136 (60%) presented with T2 tumors, 188 (83%) with N0/1 and 128 (56.6%) with estrogen receptor negative BC. In details, basal TRAR score was available for 69, 79 and 78 patients assigned to neoadjuvant trastuzumab, lapatinib, and their combination, respectively. Overall, patients achieving a pCR showed significantly lower levels of TRAR score than those with residual disease (p <.0001). According to treatment arm, the classifier highly performed in discriminating responders (pCR) from non responders (no pCR) (trastuzumab: AUC = 0.74, 95% CI: 0.60–0.88; lapatinib: AUC = 0.76, 95% CI: 0.65–0.87; trastuzumab + lapatinib: AUC = 0.71, 95% CI: 0.59–0.83). Of note, the predictive value of the TRAR score was confirmed after adjustment for hormone receptor status. An AUC value of 0.70 (95% CI: 0.59–0.81) and 0.71 (95% CI: 0.62–0.80) was obtained in estrogen receptor positive and negative patients, respectively. We also evaluated the contribution to the predictive capability of TRAR levels and PAM50 by implementing a multivariate logistic regression model including both classifiers and clinically relevant variables. In this model the most predictive variable was TRAR. At a median follow-up of 7 years, with a total of 64 events, no statistically significant association was found between the TRAR score and EFS.

CONCLUSION: Overall, we show that our 41-gene signature is accurate in predicting patient response to neoadjuvant HER2 targeted therapy in terms of pCR. In particular, low levels of TRAR score can identify a HER2-positive breast cancer subgroup highly responsive to trastuzumab as monotherapy for whom combination with other HER2-targeted drugs does not appear justified and may be one tool used for exploring de-escalating strategies without sacrificing outcomes.
Title: Association of intrinsic subtype and immune genes with pathological complete response in the OPTIHER-HEART phase II clinical trial following neoadjuvant trastuzumab/pertuzumab-based chemotherapy in HER2-positive breast cancer

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Body: Background. In HER2-positive early breast cancer, neoadjuvant therapy based on dual HER2 blockade with trastuzumab and pertuzumab in combination with chemotherapy is associated with high rates of pCR. The OPTIHER-HEART trial examined the safety of the neoadjuvant dual blockade added to paclitaxel and liposomal doxorubicin (Gávila et al, SABCS 2016). In this analysis, we examined the intrinsic subtypes and immune genes in baseline and surgical samples from the OPTIHER-HEART trial.

Methods. OPTIHER-HEART was a multicenter single-arm phase II study of 18 weeks of neoadjuvant pertuzumab, weekly trastuzumab, liposomal doxorubicin (50 mg/m2 every 21 days) and weekly paclitaxel 80 mg/m2 in patients with stage II-IIIB HER2-positive breast cancer. pCR was defined as absence of infiltrating tumor in the breast and axilla. The expression of 55 breast cancer-related and immune genes in baseline samples and surgical specimens was measured using the nCounter platform. Intrinsic subtypes were determined by the PAM50 gene expression predictor. Univariate and multivariable logistic regression models adjusted for tumor size, ER status, age and nodal status was performed.

Results. A total of 58 of the 83 (69.8%) baseline tumor samples were available. 51.7% (n=30) were classified as HER2 enriched (HER2-E) followed by Normal-like (15.5%; n=9), Basal-like (12.0%; n=7), Luminal A (10.3%; n=6), and Luminal B (10.3%; n=6). HER2-E was the most frequent subtype in both hormone receptor (HR)-negative (50.0%) and HR-positive (52.0%) tumors. The pCR rates varied according to intrinsic subtype (p<0.001). The highest rate of pCR was observed in Basal-like (85.7%) and HER2-E (83.3%), followed by Normal-like (44.4%), Luminal A (33.3%) and Luminal B (16.6%). HER2-E tumors were associated with higher pCR rates compared to non-HER2-E tumors (83.3% vs 46.5%, unadjusted odds ratio = 5.76, 95% CI 1.71 – 19.42, P=0.004), even after adjusting for HR status, size, age and lymph node involvement (odds ratio = 13.50, 95% CI 2.51 – 72.52, P=0.002). Among the 55 genes, 14 (25.5%) were statistically significantly associated with pCR in univariate analysis (false discovery rate < 1%). Genes associated with pCR were 17q12-21 amplicon genes (e.g. GRB7 and ERBB2) and immune genes (e.g. CD8A and PD1). Genes associated with residual disease were luminal-related genes (e.g. ESR1 and PGR). Among the 14 genes, only 7 (i.e. CDH3, CD8A, PD1, EGFR, ESR1, SLC39A6, NAT1) were significantly associated with pCR beyond intrinsic subtype (HER2-E vs non-HER2-E). Finally, a total of 58 of the 80 (72.5%) surgical specimens were profiled. 77% of the samples were classified as Normal-like, followed by Luminal A (20%), Luminal B (2%) and HER2-E (2%). Normal-like was identified in a higher proportion in pCR samples (90%) compared to samples with residual disease (47%) (p<0.001).

Conclusion. HER2-E subtype and immune genes are independently associated with pCR after dual HER2 blockade and multi-agent chemotherapy. Subtyping of surgical specimens might provide additional response and outcome data and warrants further evaluation.
Title: Independent validation of the PAM50-based chemo-endocrine score (CES) in hormonal receptor positive (HR+)/HER2+ breast cancer (BC) treated with neoadjuvant (NA) anti-HER2-based therapy

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Body: Background. HER2+/HR+ BC is heterogeneous and subgroups with different treatment sensitivities need to be identified. We previously reported a PAM50-based CES in HR+/HER2-negative BC (Prat et al. CCR 2016). Here, we evaluated the association of CES with pathologic complete response (pCR) following anti-HER2-based therapy in HR+/HER2+ BC across 6 NA studies.

Methods. Intrinsic subtype and clinico-pathological data were obtained from 6 NA clinical studies (CHERLOB, OptiHERHEART[OHH], PAMELA, LPT109096, ICO and CALGB 40601 [CALGB is part of the Alliance for Clinical Trials in Oncology]). All patients (pts) received chemotherapy (CT) and trastuzumab, except for pts in the PAMELA study who did not receive CT. A second anti-HER2 agent (lapatinib or pertuzumab) was included in the NA of all pts in OHH and PAMELA and in one of the treatment arms in CHERLOB, LPT and C40601. CES was evaluated as a continuous variable, and categorically (CES-E[endocrine-sensitive], CES-U[uncertain] and CES-C[chemo-sensitive]) using the previously reported cut-offs. In all studies, except CHERLOB and C40601, ERBB2 mRNA levels were measured using the nCounter platform. pCR in the breast was the endpoint. We first performed statistical analyses in each dataset individually, and then in a dataset with combined patient-level data. Univariate and multivariable logistic regressions analyses were used.

Results. A total of 345 pts were included in the analysis. In the combined cohort, CES-E, CES-U and CES-C were identified in 27.5%, 23.7% and 48.6% of the pts, respectively. Table 1 summarizes the distribution and pCR rates by CES groups. In the combined cohort, CES-C was associated with higher pCR rates compared to CES-U and CES-E independent of tumor size, nodal status, anti-HER2 treatment (single vs. dual HER2 blockade), intrinsic subtype (HER2-enriched [HER2E] versus not) and study (Table 2). In the PAMELA trial (no CT), CES-C was also found associated with higher pCR rates compared to CES-E (31.6% vs. 0%). Concordant with this finding, CES-C tumors showed higher ERBB2 mRNA levels than non-CES-C (P<0.01).

Conclusion. CES shows clinical validity for predicting CT/HER2-targeting sensitivity in HER2+/HR+ BC beyond intrinsic subtype and clinicopathologic characteristics. HER2+/HR+/CES-E tumors, which represent ~20% of all HER2+ tumors, show low sensitivity to anti-HER2 regimens (with and without CT); other treatment strategies might be needed for this group.

Support: U10CA180882;U10CA180821;U24CA196171;P50-CA58823;BCRF;Komen.

Table 1. Distribution and pCR rates of CES-E, CES-U and CES-C groups across studies.
Table 2. Association of CES, intrinsic subtype, clinicopathologic variables and study with pCR in a multivariable model (including type of study).

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Title: Quantitative spatial profiling of tumor associated macrophages and the PD-1/PD-L1 interaction in breast cancer

Vasiliki Pelekanou1, Veronique Neumeister1, Lajos Pusztai1 and David L Rimm1. 1Yale School of Medicine, New Haven, CT.

Body: Background: Although immunotherapy approaches are being successfully administered in some breast cancer (BC) patients (pts), biomarkers of response remain elusive. Tumor associated macrophages (TAMs) are the most prominent immune cells in breast tumors, mediating the cross-talk between tumor cells and tumor infiltrating lymphocytes (TILs). Specific biomarkers of breast TAMs’ functional status remain to be defined. CSF-1R, is a TAMs regulator and key target across cancers clinically tested, alone or combined with anti-PD-1 checkpoint inhibitors. The goals of the study were: 1) to objectively measure CSF-1R expression within all CD68+ and M2-like CD163+TAMs, as well as PD-L1/PD-1 spatial interaction and 2) to determine whether objectively quantifying these key immune mechanisms related to TAMs immunomodulation within the tumor microenvironment can predict outcome and potentially response to immunotherapy.

Methods: Tissue Microarrays (TMAs) from two Yale BC cohorts (Cohort A, all breast cases, n=320) (Cohort B, TNBC, n=132) were assessed by quantitative immunofluorescence (QIF) for CSF-1R/CD163/CD68; PD-1/PD-L1 interaction score (proportion of PD-1+ cells co-localized with PD-L1) and co-expression of the multiplexed biomarker panels. Biomarker positive cells and their co-localization were objectively measured using the AQUA method of QIF. QIF scores were compared by linear regression coefficients ($R^2$). Overall and recurrence-free survival (OS and RFS) were assessed. Our protein data were compared with transcriptome data from the METABRIC study obtained from www.cbioportal.org.

Results: CSF-1R expression was associated with expression of both CD68 and CD163 in both cohorts (A: $R^2=0.64$, B: $R^2=0.49$). CSF-1R in CD163/CD68 was higher in TNBC Cohort B (p<0.01) and in ER- cases of Cohort A (p=0.004). In Cohort A high CSF-1R expression (top 10%) in CD163+/CD68+ cells was associated with worse OS and RFS (All cases, p=0.015 and p=0.0005, respectively). After ER-status adjustment, high CSF-1R (in CD68 and CD163) was associated with worse OS and RFS only in ER- cases of Cohort A (p=0.004 and p=0.0004) and increased recurrence rate (p=0.009). High CSF-1R/CD163 (top 10%) was also associated with increased recurrence rate (p=0.004). In TNBC, high CSF-1R correlated with worse OS (p=0.01) only in CD163+ TAMs. High CSF-1R scored as a continuous variable was related with worse RFS in both CD68+ (p=0.0026, RR 1.00/2.86) and CD163+ TAMs (p=0.006, RR 1.00/2.76). However, in multivariate analysis CSF-1R was not an independent prognostic factor for OS or RFS.

PD-L1 mostly co-localized with CD68 TAMs ($R^2=0.7$). Tumor PD-L1 tended to be mutually exclusive of CSF-1R. PD-L1/PD-1 colocalization was higher in TNBC (p<0.01) and associated with better OS (p=0.01). CSF-1R in TAMs tended to be higher when PD-1/PD-L1 colocalization was low.

The trend of mutual exclusivity between CSF-1R in TAMs and PD-1/PD-L1 was confirmed by expression (mRNA) data from METABRIC study.

Discussion: This novel multiplexed method profiling key tumor-immune suppression pathways could identify BC pts likely to respond to anti-PD-1/anti-CSF-1R therapy. This method could help stratify pts for mono- or combined therapy in future clinical trials.
Title: Low levels of HER2 extracellular domain (ECD) compared to intracellular domain (ICD) in NeoALTTO may segregate benefit from lapatinib and trastuzumab in breast cancer

Body: Background: Preclinical models suggest that in some HER2 overexpressed breast cancer cases, the extracellular domain of HER2, containing the binding region for trastuzumab (T) is not present due to proteolytic cleavage. Previous measurement of HER2 ECD and ICD in HeCOG 10/05 suggested that breast cancer cases that have low levels of ECD show decreased benefit from adjuvant T therapy (Carvajal et al, 2015). These cases may be more likely to show benefit from lapatinib (L), a small molecular tyrosine kinase inhibitor. NeoALTTO, an international multi-institutional trial comparing pre-surgical treatment with L vs T vs both, showed that pCR rates were around 25-30% for L or T but increased to 50% for both. This cohort allows us to test the hypothesis that patients with low ECD may benefit more from L than T.

Methods: ECD and ICD were measured 382 cases using quantitative immunofluorescence (QIF) with domain specific antibodies (SP3, Spring biosciences for ECD and CB11, Biocare for ICD). Slides were stained and scanned, then measured using the AQUA method of QIF. All fields of view were scored and FOVs were averaged to generate a QIF score for each case. Index TMAs were used to standardize all autostainer runs and to define the QIF score cut-point that is equivalent to the clinical cut-point for the index TMA cases. Each case was then assigned to one of three groups; 1) High ECD and ICD, 2) Low ECD but High ICD, and 3) low ICD.

Results: In the lapatinib arm, both group 1 and 2 showed a pCR rate of 25% (p>0.99), but in the trastuzumab arm, Group 1 pCR rate was 34% compared to 19% for group 2 (p=0.38). In the combination arm, group 1 showed a pCR rate of 59% compared to 29% for group 2 (p=0.046). In a logistic regression model for pCR, after adjustment for treatment arm and HR status the ECD/ICD status shows a significant predictive value (p = 0.002). Although this study is not powered for event free survival (EFS), at 6 years of follow up we found that patients in group 2 on the lapatinib arm show 92% EFS compared to 69% EFS in group 1 (p=0.17). The opposite trend is seen in both trastuzumab arms where EFS for group 1 on T showed 70% vs 62% for group 2 (p=0.57) and 77% vs 71% on the T+L arm (p=0.74). In a Cox regression model for EFS after adjustment for treatment arm and HR status, the ECD/ICD status is not significant (p=0.88)

Conclusions: Cases with low levels of ECD compared to ICD appear benefit less than those with high levels of both ECD and ICD as assessed by pCR from treatments containing T. In contrast, those same patients also appear to benefit more, as assessed by EFS, on the L arm. These observations are consistent with the hypothesis that this assay could stratify benefit from L vs T based on the status of the ECD. Further assessment using this assay on the ALTTO cases is currently underway and may help confirm this observation.
2017 San Antonio Breast Cancer Symposium

Title: Analysis of biomarkers for response and resistance to the AKT inhibitor MK-2206 in the neoadjuvant I-SPY 2 trial for stage II-III high-risk breast cancer

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Body: Background: The AKT inhibitor MK2206 (M) was one of the experimental agents evaluated in I-SPY 2, and graduated in the HER2+, HR-, and HR-HER2+ signatures. In I-SPY 2, all patients received at least standard chemotherapy (paclitaxel followed by doxorubicin/cyclophosphamide; T->AC). HER2- patients were randomized to receive M+T- >AC vs. T->AC. For HER2+ patients, M was administered in combination with trastuzumab (M+H+T->AC vs. H+T->AC). We hypothesize that genes in the AKT signaling axis may specifically predict response to M and tested expression levels of 10 genes: AKT1, EGFR, ERBB2, ERBB3, NRG1, IGF1R, PIK3CA, PTEN, STMN1, and MTOR. We also evaluated 9 additional genes previously shown to associate with response to M in vitro and through exploratory analyses in the metastatic setting: STARD3, TM7SF2, ALDH4A1, PRODH, SELENBP1, G3BP1, SMCR7L, TCTEXD2, and PHEX.

Methods: Data from 150 patients (M: 94 and concurrent controls: 56) were available. Pre-treatment biopsies were assayed using Agilent 44K (32627; n=119) or 32K (15746; n=31) expression arrays; and these data were combined into a single gene-level dataset after batch-adjusting using ComBat. All I-SPY 2 qualifying biomarker analyses follow a pre-specified analysis plan. We used logistic modeling to assess biomarker performance. A biomarker is considered a specific predictor of M response if it associates with response in the M arm but not the control arm, and if the biomarker x treatment interaction is significant (likelihood ratio test, p<0.05). This analysis is also performed adjusting for HR and HER2 status as covariates, and within receptor subsets, sample size permitting. Our statistics are descriptive rather than inferential and do not adjust for multiplicities of other biomarkers outside this study.

Results: Consistent with M graduation in the HER2+ signature, two candidate biomarkers on the HER2 amplicon (ERBB2, STARD3) associate with pCR in the M arm, but not in the control arm. In addition, G3BP1, a component of the RAS signaling pathway, associates with non-pCR in the M arm. However, biomarker x treatment interactions for these genes are not significant, and all three associations to response in M lose significance in a model adjusting for HR and HER2 status. Within the HER2+ subset, IGF1R is associated with non-pCR in M. Within the TN subset, higher levels of NRG1 and PIK3CA, upstream activators of AKT, associate with pCR in the M arm.

Conclusion: Following our pre-specified analysis, none of the candidate markers tested succeed as specific predictors of response to MK2206 in I-SPY 2. However, several genes in the AKT pathway associate with response to M, and in particular PIK3CA levels within the TN subset may merit further evaluation in future trials.
Title: Polyligand profiling differentiates cancer patients according to their benefit of treatment

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Body: Introduction: Deconvolution of multi-nodal perturbations in cancer network architecture demands highly multiplexed profiling assays. We demonstrate the value of polyligand profiling of tumor systems states using libraries of single stranded oligodeoxynucleotides (ssODN) to distinguish between tumor tissue from breast cancer patients who did or did not derive benefit from treatment regimens containing trastuzumab.

Methods: This study included cases from women with invasive breast cancer who received chemotherapy+ trastuzumab (C+T) or trastuzumab monotherapy with available retrospective data on the time to next treatment (TTNT). A library of 2x10^{12} unique ssODN was exposed to FFPE tissues from patients who benefited (B) or not (NB) from trastuzumab-based regimens in several rounds of positive and negative selection. Two enriched libraries were screened on independent set of 42 B and 19 NB cases using a modified IHC protocol for detection of bound ssODNs. Poly-Ligand Profiles (PLP) were scored by a blinded pathologist. Two libraries, EL-NB and EL-B, showed significant p-values between groups of responders and non-responders. A Cox-PH model was fitted using either tumors’ HER2 status or PLP test results as the independent variable. Median survival time was calculated from the Kaplan-Meier estimate. A separate group of 63 cases with TTNT data from chemotherapy without trastuzumab was used as a control to distinguish prognostic from predictive performance.

Results: The PLP scores of EL-NB and EL-B were assessed by receiver operating characteristic (ROC) curves and resulted in a combined AUC value of 0.81. EL-NB and EL-B were able to effectively classify B and NB patients with either HER2-negative/equivocal (AUC = 0.73) or HER2-positive cancers (AUC = 0.84). In contrast, HER2 status alone yielded an AUC value of 0.47. The combined PLP scores for the independent set of 63 patients treated with C excluding trastuzumab resulted in an AUC value of 0.53, indicating that the assay was predictive and not simply prognostic. Kaplan-Meier curves analysis shows that PLP+ cases have 429 days median TTNT, while PLP- cases have 129 days (HR = 0.38, log-rank p = 0.001). Analysis based on HER2 status showed no significant difference in TTNT between patients that were HER2+ (280 days) or HER2-negative/equivocal (336 days, HR = 1.27, log-rank p =0.45).

Summary: Performance of the PLP assay in differentiating patients who did or did not benefit from trastuzumab therapy outperforms the standard IHC assay for HER2 status. These results represent a promising step towards the development of a CDx to identify the 50-70% of HER2+ patients who will not benefit from trastuzumab. In addition, PLP also has the potential to identify the HER2-negative/equivocal patients who may benefit from trastuzumab-containing regimens.
Title: Comprehensive gene expression biomarker analysis of CDK 4/6 and endocrine pathways from the PALOMA-2 study

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Body: Background: Palbociclib (P) is a potent, oral, highly selective, inhibitor of cyclin-dependent kinase (CDK) 4 and 6. In the Phase 3 PALOMA-2 study, P in combination with letrozole (L) demonstrated a significant improvement in progression-free survival (PFS) versus placebo (PBO) plus L. To further explore predictive markers for sensitivity and resistance to CDK4/6-based therapy or endocrine therapy (ET) alone, we performed a large gene expression analysis of baseline tumor tissues using mature PFS data with extended follow-up.

Methods: Postmenopausal women (N=666) with no prior systemic therapy for ER+/HER2- ABC were randomized 2:1 to P+L or PBO+L until disease progression, death, unacceptable toxicity, or consent withdrawal. All patients provided tumor tissues to participate in the study. 455 of 666 intent-to-treat (ITT) patients had tumor tissue appropriate for gene expression evaluation (303 [68%] P+L arm and 152 [68%] PBO+L arm). EdgeSeq Oncology Biomarker Panel (HTG Molecular Diagnostics; Tucson, AZ) was used for mRNA profiling, assessing 2534 genes involved in a variety of cancer-related pathways. Using systematic and unsupervised approaches, gene expression levels were interrogated for identification of potential genes and/or pathways associated with a treatment effect of P+L compared with PBO+L.

Results: With median follow-up of 38 months, mPFS of P+L vs L + PBO was 27.6 mos vs 14.5 mos (HR=0.563 [p<0.000001] in ITT). PFS in the biomarker-assessed group was similar. Using a supervised approach, expression of genes involved in the Cyclin D-CDK4/6-RB pathway were analyzed. This demonstrated that patients receiving P+L had a consistent benefit similar to the ITT population irrespective of various expression levels of CCND1, CCNE1/2, CDK2/4/6, RB1, and CDKN2A. These results are consistent with findings from previous IHC data (CCND1, RB, p16). Unsupervised analysis revealed that tumors with higher levels of growth factor receptors (eg. FGFR2 FDR 0.032, interaction p=0.056 and ERBB3 FDR 0.221, interaction p=0.043,) were associated with greater sensitivity to P+L vs L alone. Using parallel gene signature-based analyses, tumors with higher expression of a growth factor signature had longer PFS in the P+L arm. In addition, higher CDK4 expression was identified as a resistance marker for PBO+L arm (FDR 0.095, interaction p=0.016). Gene expression-based molecular subtyping demonstrated that both luminal A and B subtypes benefited equally from P+L vs PBO+L. Patients with lower level of tumor PD-1 expression showed more benefit from P+L (FDR 0.099, interaction p=0.021).

Conclusion: These results confirm efficacy of palbociclib + letrozole in ER+/HER2- breast cancer, and support the use of ER+ as a biomarker for sensitivity to CDK 4/6 inhibition. Expression levels (whether high or low) of genes in the Cyclin D-CDK4/6-RB pathway did not correlate with benefit from palbociclib + letrozole. These data provide evidence that the interplay between steroid hormone and peptide growth factor signaling in ER+ breast cancer drives dependence on CDK 4/6 and benefit from CDK 4/6 inhibition with palbociclib. These data can be used to guide additional therapeutic opportunities in ER+/HER2- ABC.

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**Title:** PAM50 intrinsic subtyping as a predictor of pathological complete response to neoadjuvant trastuzumab-based chemotherapy in early HER2-positive breast cancer

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**Body:**

**Background:** HER2-positive breast cancer (BC) is a heterogeneous disease from a clinical and biological perspective. Intrinsic subtype defined by gene expression has an important role in determining response to treatment, as seen in several neoadjuvant trials (e.g. CALGB40601, CherLOB, NeoALTTO and PAMELA). However, limited data exist in an off-trial setting. The objective of this study was to evaluate the association of intrinsic subtypes with pathological completed response (pCR) and survival outcomes of a series of HER2-positive patients (pts) homogeneously treated with trastuzumab-based primary chemotherapy (PC) in a single comprehensive cancer center.

**Methods:** Clinical-pathological data were evaluated in a series of 150 consecutive stage II-IIIC (T4d included) HER2-positive BC pts treated in ICO-Hospitalet (Spain) from August-2009 to December-2012 with weekly paclitaxel x12 followed by FEC/3w x 4 and concurrent trastuzumab for a total of 24w. HER2-positivity was considered according to ASCO-CAP 2007 guidelines. pCR was defined as ypT0/isypN0. The expression of 105 BC-related genes, including the PAM50 genes, was determined in baseline and residual formalin-fixed paraffin-embedded tumor samples using the nCounter platform. Intrinsic subtypes were determined by the research-based PAM50 gene expression predictor. Association of variables with pCR or disease-free survival (DFS) was evaluated using logistic regression analyses and cox proportional hazard models. All statistical tests were two-sided and considered significant when \( p \leq 0.05 \).

**Results:** Most pts had T2 (64%) and T4 (20%) tumors and clinically node-positive disease (77%). 53% had hormonal receptor (HR)+ disease. 84 of the 150pts (56%) achieved a pCR; HR-neg was associated with higher pCR rates (72.5% vs 42% in HR+ \( p<0.001 \)). 90 of the 150 (60%) baseline samples were evaluated. Baseline subtype distribution: HER2-enriched (HER2-E) 63%, Luminal A 11%, Basal-like 8.9%, Normal-like 8.9% and Luminal B 7.8%. Although HER2-E predominated in HR-neg tumors (74%), 53% of HR+ tumors were HER2-E. pCR rates varied according to intrinsic subtype (\( p<0.001 \)). HER2-E tumors were associated with higher pCR rates compared to non-HER2-E (68.4% vs 33.3%, \( p<0.001 \)) regardless HR-status. Five of the 8 PAM50 signatures (HER2E, ROR-S, ROR-P, Basal-like and Proliferation score) were associated with pCR, whereas Luminal A was associated with no-pCR (\( p<0.001 \)). With a median follow-up of 6.6 years, HER2-E subtype was associated with a better DFS compared to non-HER2-E (5-year DFS 92.4% vs 75.9%; HR= 0.27; 95% CI 0.08-0.91; \( p=0.034 \)). Finally, 28 of the 66 (42.4%) surgical specimens with residual disease were studied. Residual subtype distribution was: Normal-like (50.0%), Luminal A (32.1%), HER2-E (14.3%) and Luminal B (3.5%).

**Conclusions:** In this consecutive series of HER2-positive BC treated homogeneously with neoadjuvant trastuzumab-based PC, all of the main intrinsic molecular subtypes were identified with a predominance of HER2-E. HER2-E was significantly associated with pCR and survival outcome. Distribution of the intrinsic subtypes in residual disease differed from untreated tumors.
**Title:** Independent validation of the HER2-enriched subtype as a predictor of pathological complete response following trastuzumab and lapatinib without chemotherapy in early-stage HER2-positive breast cancer

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**Body:**

**Background:** HER2-positive breast cancer consists of 4 intrinsic molecular subtypes (Luminal A, Luminal B, HER2-enriched [HER2-E], and Basal-like) and a Normal-like subtype, with the HER2-E subtype having the highest activation of the EGFR-HER2 pathway. Concordant with this, the HER2-E subtype was significantly associated with pathological complete response (pCR) following lapatinib and trastuzumab without chemotherapy on the PAMELA phase II neoadjuvant trial (Lancet Oncol 2017). Here, we aimed to further validate this observation in a different cohort using tumor samples from the TBCRC023 trial.

**Methods:** TBCRC023 (NCT00999804) was a randomized phase II trial combining a Simon Phase 2 design in the experimental arm with a pick-the-winner design, not powered for direct comparison. Ninety-seven women with HER2+ breast cancer measuring 2 cm or larger (median = 5 cm) were randomized in a 1:2 ratio to 12 vs. 24 weeks of lapatinib and trastuzumab. Letrozole (along with ovarian suppression if premenopausal) was added in patients whose tumors were also estrogen receptor (ER)-positive. All evaluable patients were assessed for pCR, defined as no residual invasive carcinoma in the breast. Intrinsic molecular subtypes from tumor biopsy formalin-fixed, paraffin-embedded samples taken at baseline (day 0) were determined with the nCounter-based PAM50 predictor. Gene expression PAM50 data was performed at Hospital Clinic in Barcelona blinded from clinical data. The primary outcome was the association of the HER2-E subtype (vs. non-HER2-E) with pCR. A logistic regression model adjusted for tumor size, ER status, nodal status and treatment arm was performed.

**Results:** A total of 85 of the 97 (87.6%) baseline tumor samples were available. Most patients had the HER2-E subtype (51 [60.0%]), followed by Normal-like (12 [14.1%]), Basal-like (11 [13.0%]), Luminal B (7 [8.2%]) and Luminal A (4 [4.7%]). The proportion of patients with HER2-E tumors within ER+ and ER-negative disease was 54.9% and 67.7%, respectively. At the time of surgery, 17 of 85 patients (20.0%; 95% confidence interval [CI] 0.13-0.30) had a pCR in the breast. Fourteen of 51 patients with the HER2-E subtype (27.5%; 95% CI 0.17 to 0.41) and 3 of 34 patients with non-HER2-E subtypes (8.8%; 95% CI 0.03 to 0.23) achieved a pCR at the time of surgery (adjusted odds ratio 4.33; 95% CI 1.08-17.41; P=0.039). No other clinical-pathological variable was found significantly associated with pCR in the multivariable model.

**Conclusions:** In an independent validation study performed while blinded to clinical outcomes, HER2-E subtype confirms its ability to identify patients with HER2-positive breast cancer who are likely to benefit from dual HER2 blockade therapies without chemotherapy. Further studies should be performed to prospectively validate this biomarker, alone or in combination with other biomarkers.
Body: Background: Metastatic triple negative breast cancer (mTNBC) has a poor prognosis with limited treatment options. Atezolizumab (atezo) is a humanized monoclonal antibody that inhibits the binding of PD-L1 to PD-1 and B7.1. Atezo has demonstrated promising activity in the mTNBC cohort of the phase 1 PCD4989g study. Clinical activity was previously linked to higher PD-L1 expression by IHC (ORR: 13% vs 5%) and TILs (ORR: 13% vs 7%) (Schmid et al., AACR 2017, NCT01375842). Here we characterize molecular features of tumors from atezo-treated patients and explore their potential association with clinical activity.

Methods & Results: Molecular characterization of pre-treatment tumor tissue was performed to investigate potential biological mechanisms associated with the clinical activity of atezo in mTNBC. DNA mutational analysis of tumors from 78 patients was performed with the Foundation Medicine One panel. Median tumor mutation burden (TMB) was 4.6 Mut/Mb (CI 95% 3.604 - 5.405). TMB was not associated with either TILs or immune biomarkers by IHC (PD-L1 or CD8) or with clinical activity (ORR, PFS or OS). The prevalence of loss of heterozygosity or mutations in TP53 or BRCA1/2 in this mTNBC cohort was 65%, 95%, and 10%, respectively. None of these genomic alterations were associated with clinical activity to atezo. RNA-Seq based analyses were performed in tissue from 96 patients. PAM50 profiling classified these tumors as 84% basal-like, 9% HER-2-enriched, 4% luminal A and 2% luminal B. TNBC subtyping (Burstein et al., CCR 2015) classified 42% of the tumors as basal-like (BL) immune activated (BLIA), 35% BL immune suppressed (BLIS), 17% luminal androgen receptor (LAR) and 2% mesenchymal (MES). Histopathology immune biomarkers TILs, PD-L1 and CD8 were highest in BLIA, intermediate in LAR and lowest in BLIS. ORRs by RECIST 1.1 were highest in BLIA and LAR, and lowest in BLIS and MES (17.5%, 18.8%, 0% and 0%, respectively). Although univariate analysis showed that the BLIA subgroup had significantly longer PFS and OS, multivariate analysis showed an association for both the BLIA and LAR subgroups with improved PFS and OS. High expression of pre-specified B- and T-cell RNA signatures were significantly associated with better ORR, PFS, and OS.

Conclusion: Comprehensive molecular characterization of tumors from this non-randomized phase 1 mTNBC cohort showed a low TMB. Mutations in BRCA1/2, TP53, or loss of heterozygosity did not impact atezo clinical activity. Clinical benefit from atezo was enriched in BLIA and LAR TNBC subtypes, both representing tumors with a rich tumor immune microenvironment.
Title: A predictive model of pathological response following dual HER2 blockade-only based on tumor cellularity and tumor-infiltrating lymphocytes (CelTIL) in HER2-positive breast cancer

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Body: Background: Increased number of tumor-infiltrating lymphocytes (TILs) at baseline is associated with pathological complete response (pCR) and improved outcomes in HER2-positive early breast cancer treated with anti-HER2-based chemotherapy. In the absence of chemotherapy, the association of TILs with pCR following anti-HER2 therapy-only is currently unknown.

Methods: The PAMELA (NCT01973660) neoadjuvant trial treated 151 women with HER2-positive breast cancer with lapatinib and trastuzumab (and hormonal therapy if hormone receptor-positive) for 18 weeks. Percentage of TILs and tumor cellularity were determined at baseline (n=148) and after 2 weeks of treatment (n=134). Associations of TILs and tumor cellularity with pCR in the breast were evaluated using univariate and multivariable logistic regression models. The regression coefficients were used to derive a score based on TILs and tumor cellularity measured at week 2 (CelTIL) was derived in PAMELA and tested in week 2 samples from 65 patients with HER2+ disease recruited in the LPT109096 (NCT00524303) phase 2 neoadjuvant trial, where anti-HER2 therapy-only (trastuzumab, lapatinib or the combination) was administered for 2 weeks, followed by the addition of standard multi-agent chemotherapy for 24 weeks.

Results: In PAMELA, at baseline, TILs were significantly associated with pCR in univariate analysis but not in multivariable analysis (adjusted odds ratio [OR]=1.01, 0.98-1.03; p-value=0.620). A statistically significant increase in TILs was observed at week 2 compared to baseline (mean difference +6.9%; p-value<0.001). At week 2, TILs were significantly associated with pCR in univariate and multivariable analyses (adjusted OR=1.04, 1.01-1.06; p-value=0.009). TILs and tumor cellularity at week 2 were independently associated with pCR and a combined score (from 0 to 100) taking into account both variables was derived. CelTIL as a continuous variable was significantly associated with pCR, and patients with CelTIL-low and CelTIL-high scores (cutoff < 33.59) had a pCR rate of 0% and 33%, respectively. Independent validation of CelTIL in week 2 samples from 65 patients with HER2+ disease recruited in the LPT109096 phase 2 neoadjuvant trial will be presented at the conference.

Conclusions: A combined score of TILs and tumor cellularity at week 2 following anti-HER2 therapy-only is associated with pCR upon completion of neoadjuvant anti-HER2-based therapy.
**2017 San Antonio Breast Cancer Symposium**

**Publication Number:** P2-09-15

**Title:** NTRK fusions in breast cancer: Clinical, pathologic and genomic findings

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**Body:**

**Background:** The tropomysin receptor kinase A family includes the 3 NTRK1, NTRK2 and NTRK3 genes and plays major roles in neuronal development. The recent evidence of remarkable efficacy for kinase inhibitors (TKI) targeting NTRK across a wide variety of malignancies that harbor NTRK gene fusions has stimulated great interest in determining the type of cancers driven by these therapy defining NTRK genomic alterations.

**Methods:** A consecutive series of 12,214 locally aggressive, relapsed and metastatic breast malignancies (mBM) were subjected to comprehensive genomic profiling (CGP) using DNA extracted from 40 µm of FFPE sections and adaptor ligation-based libraries to a mean coverage depth 719X for up to 315 cancer-related genes. The results were analyzed for all classes of genomic alterations (GA) including base substitutions, insertions and deletions, select fusions and rearrangements, and copy number changes. Tumor mutational burden (TMB) was determined on 1.1 Mbp of sequenced DNA. Microsatellite instability (MSI) status was calculated by a customized algorithm.

**Results:** 16 (0.13%) mBM (all female) harbored NTRK gene fusions. The median age was 51 years (range 34 to 70 years). There were 9 ductal carcinomas, 2 lobular carcinomas, 3 secretory carcinomas 1 metaplastic carcinoma and 1 angiosarcoma. Tumor stages at the time of sequencing were 12 Stage IV, 1 Stage III and 3 stage I (all 3 secretory carcinomas). In 9 cases, clinical receptor status was known: 3 (33%) were ER+/HER2- and 6 (66%) ER-/HER2- (all TNBC) with all 9 (100%) of cases HER2-. All 3 SCA were TNBC. 10 NTRK fusions involved NTRK1 featuring a variety of fusion partners (CGN, GATAD2B, LMNA, MDM4, PEAR1, and TPM3) and 6 involved NTRK3 (all ETV6 fusions). There were no NTRK2 fusions. NTRK fusion+ mBM featured a mean of 4.25 GA per sample. The most frequent non-fusion partner co-altered genes in this series of NTRK fusion+ mBM were: TP53 at 25%, IKBKE, PIK3C2B, CCND1 at 19%, and AKT, PIK3CA, MYC, CDH1, CDKN1A, PTEN, FGF3, FGF4 and FGF19 all at 13%. The median TMB for NTRK fusion+ mBM was 0.9 mutations/Mb and no cases (0%) had a TMB ≥ 10 mutations/Mb and no cases (0%) feared high microsatellite instability (MSI high). Clinical response assessment to NTRK TKI therapies in this series is ongoing.

**Conclusions:** NTRK gene fusions although extremely uncommon in breast malignancies occur across a variety of tumor types, is universally HER2 negative, more frequent in TNBC than in ER+ tumors, is associated with a moderate frequency of additional genomic alterations and a complete absence of either high TMB or high MSI. This study confirms that a CGP assay, when applied to a large cohort of near universal clinically advanced disease can identify extremely rare alterations that can lead a small number of patients to highly effective precision therapies.
Title: Tumor infiltrating lymphocytes (TILs) as a biomarker for resistance to palbociclib (Pal) in the NeoPalAna trial

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Body: Background: Cyclin-dependent kinase (CDK) 4/6 inhibitors improve disease free survival for patients (pts) with advanced hormone receptor positive (HR+) and HER2 negative (HER2-) breast cancer (BC). However, there are no established biomarkers that identify sensitive versus resistant tumors. We have recently reported results from the phase II neoadjuvant NeoPalAna trial (NCT01723774) which demonstrated that Pal enhanced the anti-proliferative activity when added to anastrozole (Ana) monotherapy in HR+HER2- BC. Interestingly, a small group of pts (15%) were resistant to Pal, exhibiting persistent tumor cell proliferation (Ki67 >2.7%) on Ana plus Pal. Several studies have evaluated the prognostic and predictive importance of TILs in BC, particularly in triple negative and HER2+ subtypes. Studies evaluating TILs in HR+ BC is limited. Here we evaluated the utility of TILs in identifying Pal-resistant tumors.

Methods: Serial biopsies were collected from pts at 4 time points: baseline (BL), cycle 1 day 1 (C1D1) following 28 days of Ana monotherapy, cycle 1 day 15 (C1D15) at 2 weeks post the addition of Pal, and at surgery (Surg). TILs were evaluated using published recommendations by the TILs international working group. Agilent 4X44 whole genome gene expression arrays performed on fresh frozen biopsies at BL, C1D1, and C1D15 were analyzed for pathways and gene signatures that differentiate Pal-resistant (Pal-r) (C1D15 Ki67 >2.7%) from Ana-sensitive (Ana-s) (C1D1 Ki67 ≤2.7%) or Pal-sensitive (Pal-s) (C1D1 Ki67 >2.7% but C1D15 Ki67 ≤2.7%) tumors defined by Ki67 response. TILs at each time point were pairwise compared between response groups using Wilcoxon rank sum test and Benjamini-Hochberg adjusted two-sided p-values were reported. Change between 2 time points within a response group was evaluated by Wilcoxon signed rank test.

Results: The TILs were significantly different between Ana-s and Pal-s groups [BL p=0.03, C1D1 p=0.01, C1D15 p=.02], as well as between Ana-s and Pal-r groups [BL p=0.03, C1D1 p=0.04, C1D15 p=0.02]. Overall Pal-r samples showed the highest TILs at all time points, while Ana-s samples showed the lowest TILs. There was no significant change between time points. Elevated TILs in Pal-r group was further supported by microarray gene expression analysis which demonstrated a large group of genes associated with MHC Class-I (15+ HLA and proteasome genes) as well as immune–inflammation pathways (18+ T cell & lymphocyte markers, signaling genes) being up regulated at BL in the Pal-r group (n=5). Pal-r samples showed a similar trend in subsequent time points although the numbers of samples were small. Many genes within this immune-inflammatory group of genes were correlated with Ki67 change at C1D15 from BL, suggestive of a potential relationship with resistance.

Conclusions: Our data shows Pal resistance was consistently associated with higher TILs at BL and post treatment, which correlated with increased expression of inflammation-immune group genes. TILs may have utility to be used as a biomarker to identify Pal resistant BC. Our data is hypothesis generating and raises the possibility of immune therapy to overcome Pal resistance in BC.
**Title:** Evaluation of the oncomine comprehensive assay for the identification of actionable mutations for therapeutic stratification from the TEAM pathology cohort

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**Body:** Large-scale sequencing initiatives have revealed a wealth of common and novel variants as well as copy-number aberrations, across different malignancies. This growing list of variants/aberrations can sometimes be matched to specific therapeutics. Such “actionable mutations/changes” hold promise for personalized treatment in the future, with treatments tailored to molecular abnormalities. Presently, women with hormone positive early breast cancer continue to experience improved survival on adjuvant anti-hormone therapy, but a significant number of women continue to progress. Therefore, there is a need to identify those women for whom current therapies are insufficient and to identify alternative therapeutic interventions. We explored the use of genetic profiling using a comprehensive solid tumor next generation sequencing (NGS) assay (the Oncomine Comprehensive Assay, OCA) to characterize early invasive breast cancer. The OCA is based on the Ion Torrent™ NGS platform and Ion AmpliSeq™ library preparation technology, coupled to the Oncomine™ Knowledgebase, for target selection, variant calling, and data annotations. The OCA includes 87 genes for hotspot mutation detection, 48 genes for full length sequencing and 43 genes for focal copy number assessment. The OCA provides a standardized informatics workflow and quality control (QC) parameters to process samples in a translational clinical research setting. To explore the application of the OCA to early invasive breast cancers, we performed a retrospective pilot study in a subset of cases from the TEAM trial. From the TEAM pathology samples, 420 were chosen in a case-control fashion, 413 samples were analyzed, 388 samples passed standard QC metrics, and 254 samples (65%) were found to contain 368 variants with Oncomine Knowledgebase annotations. Briefly, variants of PIK3CA were most frequent at 42.7% (157/368), followed by TP53 at 27.2% (100/368), PTEN at 5.7% (21/368), BRCA2 at 3.8% (14/368), SF3B1 (12/368), AKT1 (11/368) and PTCH1 (11/368) at 3.3%, 3.0%, 3.0%; respectively. Other variants were detected in ATM, ERBB2, RB1, FGFR2, NF1, CDKN2A, PIK3R1 and others. Amongst the 43 genes assessed for copy-number, 23 showed copy-number changes across 132 samples totalling 167 CNVs. 256 samples showed no copy-number alterations in any of the genes on the panel. ERBB2 was most frequently altered at 28.1% (47/167), followed by FGFR1 at 23.4% (39/167), CCND1 at 15.0% (25/167) and MDM2 at 10.2% (17/167). Copy-number losses were identified in TP53, RB1, PTEN, BRCA2 at 0.6% each; as well as CDKN2A at 1.8% (3/167). Analytical validation of a subset of gene variants and copy-number changes will be presented in addition to the evidence of potential future application of the Oncomine Comprehensive Assay to precision oncology goals.
Title: Multiplexed (18-Plex) measurement of protein targets in trastuzumab-treated patients using imaging mass cytometry

Body: Introduction: Recent studies have shown that the molecular heterogeneity of HER2 intracellular (ICD) and extracellular (ECD) domains, as well as overall immune infiltration, are associated with response to adjuvant trastuzumab. Traditional strategies for in situ measurement in the tumor microenvironment allow the combination of up to 6 targets, limiting our capability for in-depth interrogation of tissues. Imaging Mass Cytometry (IMC) uses metal-conjugated antibodies to provide multidimensional, objective measurement of protein targets. We used this high-throughput multiplexing platform to perform an 18-plex assessment of HER2 ICD/ECD, cytotoxic T cell infiltration and other structural and signaling proteins in a cohort of patients treated with trastuzumab.

Methods: An antibody panel for detection of 18 targets (Pancytokeratin, HER2 ICD, HER2 ECD, CD8, vimentin, cytokeratin 7, beta-catenin, HER3, MET, EGFR, ERK 1-2, MEK 1-2, PTEN, PI3K p110 alpha, Akt, mTOR, Ki67 and Histone H3) was conjugated to unique metals for detection in an IMC instrument (Fluidigm). All assays were objectively standardized and validated using quantitative immunofluoresce (QIF). Finally, the IMC technique was validated against HER2 single marker assays by QIF. We used a collection of trastuzumab-treated patients from the HeCOG 10/05 trial (n=180), and identified a case:control series using 5-year recurrence events (n=19), which were matched to controls (n=41) by age and TNM stage. Formalin-fixed, paraffin embedded tissues in tissue microarray format were ablated in the IMC attachment to the CyTOF flow cytometer for simultaneous detection of markers. Image visualization was conducted using MCD Viewer (Fluidigm). Statistical analyses were performed using a range of platforms.

Results: Patients that recurred after adjuvant treatment with trastuzumab showed a decreased fraction of HER2 ECD pixels over threshold in a compartment determined by CK and HER2 ICD compared to cases without recurrence (p=0.057). After exclusion of the lowest HER2 expressers (that would have fallen below the threshold for positive by current HER2 assays), 5-year recurrence events where associated with reduced total ECD/ICD ratio intensity in tumor (p=0.044). Patients below the median for total ECD/ICD ratio showed a trend for decreased benefit from trastuzumab (p=0.066). Levels of cytotoxic T cell infiltration, depicted by total CD8 intensity, were lower in patients with recurrences (p=0.05).

Conclusion: Objective measurement of highly multiplexed protein targets in routine, fixed breast cancer tissues shows that a decreased ratio of HER2 ECD/ICD is associated with 5-year recurrence after trastuzumab treatment. This observation is consistent with our previous work using QIF but represents the first time this has been done on identical cell content (on a single tissue section). Additionally, on the same section we found that lower levels of overall cytotoxic T cell infiltration were associated with worse outcome. Further analysis of the multiplexed data, including both correlative and distance-based analyses are underway.
**Title:** Genomic biomarker for resistance to palbociclib in the NeoPalAna trial

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**Body:**

**Background:** Cyclin-dependent kinase (CDK) 4/6 inhibitors are being evaluated in the adjuvant setting for patients with resected early stage hormone receptor positive (HR+) and HER2 negative (HER2-) breast cancer (BC). However, biomarkers that predict benefit from this class of agents are unknown. We have recently reported results from the phase II neoadjuvant NeoPalAna trial which demonstrated that palbociclib (Pal) enhanced the anti-proliferative activity when added upon anastrozole (Ana) monotherapy in estrogen receptor (ER) positive and HER2 negative breast cancers. Interestingly, a small group of patients was resistant to Pal, exhibiting persistent tumor cell proliferation (Ki67 >2.7%) on the combination of Ana and Pal. In this study, we evaluated the utility of a research algorithm for the 70-gene signature (70-GS) in identifying Pal resistant versus sensitive patients.

**Methods:** Serial biopsies were collected from patients at four treatment timepoints: baseline (BL), cycle 1 day 1 (C1D1) following 28 days of Ana monotherapy, cycle 1 day 15 (C1D15) at 2 weeks post the addition of Pal, and at surgery (Surg). RNA was extracted from frozen tumor biopsies at each timepoint and run on Agilent full genome microarrays (GSE93204) at Washington University. As an exploratory analysis, genes from the GPL8253 array that match the 70-GS were used to calculate a research approximation of the 70-GS index (r-GS). The distribution of the r-GS across Ki67 response groups was evaluated.

**Results:** Ki67 had previously been measured at each timepoint, and used to classify patients as being either Ana-sensitive (C1D1 Ki67 ≤2.7%), Pal-sensitive (C1D1 Ki67 >2.7%, C1D15 Ki67 ≤2.7%), or Pal-resistant (C1D15 Ki67 >2.7%). The r-GS was differentially regulated between sensitive (Ana or Pal) and Pal-resistant groups at BL (p=0.012), C1D1 (p=0.039), and C1D15 (p=0.022). The r-GS values varied widely across patients at BL, and generally became more positive (more low risk) with treatment. There was no correlation between Ki67 levels and r-GS. Furthermore, gene expression analysis was performed to elucidate the difference between Pal-sensitive vs. Pal-resistant patients, and Ana-sensitive vs. Pal-sensitive patients.

**Conclusions:** While on-treatment Ki67 indicated drug responsiveness, baseline r-GS significantly stratified patients into sensitive (Ana or Pal) versus Pal-resistant groups in the neoadjuvant setting. This preliminary finding suggests that the 70-GS may have clinical utility in identifying patients resistant to Pal for future studies. Additionally, results of the gene expression analysis may help to further develop genomic biomarkers for Pal and Ana sensitivity and resistance.
Title: Biomarker analysis of the LOTUS trial of first-line ipatasertib (IPAT) + paclitaxel (PAC) in metastatic triple-negative breast cancer (TNBC)

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Body: Background: The oral Akt inhibitor IPAT is being evaluated in cancers with a high prevalence of PI3K/Akt pathway activation. In the placebo-controlled randomized phase II LOTUS trial (NCT02162719), adding IPAT to PAC as first-line therapy for metastatic TNBC improved progression-free survival (PFS) in unselected patients (hazard ratio [HR]: 0.60 [95% CI: 0.37–0.98]), with a more pronounced effect in patients with PIK3CA/AKT1/PTEN-altered tumors (HR: 0.44 [95% CI: 0.20–0.99]) [Kim, Lancet Oncol in press]. An exploratory analysis was performed to understand better the potential associations between PIK3CA/AKT1/PTEN alterations and other biomarkers relevant to TNBC, as well as IPAT efficacy.

Methods: Pretreatment tumor samples (76 primary, 27 metastatic) were evaluated for genetic alterations using the FoundationOne® (Foundation Medicine) assay (n=103) and gene expression by RNA sequencing (n=73). Tumor-infiltrating lymphocytes (TILs) were quantified using the Salgado method [Salgado, Ann Oncol 2015] (n=118). Samples were classified into subtypes by gene expression based on the method developed by Lehmann and Pietenpol [Lehmann, J Clin Invest 2011].

Results: Of 42 patients (41%) with PIK3CA/AKT1/PTEN-altered tumors, 26 had an activating mutation in PIK3CA or AKT1 and 16 had an alteration in PTEN. Patients with PIK3CA- and AKT1-mutant tumors were enriched in the BL2 and LAR TNBC subtypes, whereas those with PTEN-altered tumors were enriched in the BL1 subtype. An internal analysis of the publicly available METABRIC dataset yielded similar results. PTEN alterations were also associated with reduced levels of stromal TILs compared with PIK3CA/AKT1-mutant and PIK3CA/AKT1/PTEN non-altered tumors. In an exploratory analysis of the 26 patients with PIK3CA/AKT1-mutant tumors, the effect of adding IPAT was particularly pronounced (PFS HR: 0.24 [95% CI: 0.06–0.83]; median PFS 12.9 months in the IPAT + PAC arm vs 5.0 months for placebo + PAC); interpretation of efficacy in patients with PTEN-altered tumors was limited by the size of the subgroup.

There was no enrichment of PIK3CA/AKT1/PTEN alterations in metastatic vs primary samples, nor in samples collected after (neo)adjuvant chemotherapy vs from chemotherapy-naïve patients. Additionally, there was no association between PIK3CA/AKT1/PTEN alterations and BRCA1/2 alterations. BRCA1/2 alterations were not associated with any differences in IPAT efficacy outcomes (PFS, objective response rate).

No association was observed between PIK3CA/AKT1/PTEN-altered status and gene signatures of immune cell infiltration/activation or tumor mutational burden. High (≥10%) vs low levels of stromal TILs showed a trend toward longer PFS in patients treated with placebo + PAC (HR: 0.74 [95% CI: 0.39–1.48]), but no difference was apparent in those treated with IPAT + PAC (HR: 1.14 [95% CI: 0.57–2.40]).

Conclusions: This retrospective exploratory biomarker analysis of the phase II LOTUS trial of IPAT in TNBC provides insight into the potential heterogeneity of disease biologies underlying PI3K/Akt pathway activation.
Molecular alterations and poziotinib, a pan-HER inhibitor efficacy in human epidermal growth factor receptor 2 (HER2) positive breast cancers: Combined exploratory biomarker analysis from phase II clinical trial of poziotinib for refractory HER2 positive breast cancer (BC) patients

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Body: Introduction: Poziotinib is a novel, pan-HER kinase inhibitor which showed potent anti-tumor activities through irreversible inhibition of HER family tyrosine kinases in preclinical and early clinical studies. Recent the open-label, multicenter phase II trial of poziotinib monotherapy evaluated that poziotinib is a new promising option for patients with HER2-positive metastatic BC who have failed more than two HER2 targeted therapy (NCT02418689). We evaluated genetic profiles of HER2-positive metastatic BC and investigated potential biomarkers of poziotinib for HER2-positive metastatic BC (MBC). Methods: All participants were diagnosed as HER2-positive BCs according to American Society of Clinical Oncology/College of American Pathologists HER2 guideline and provided tissue specimens that would be possible to extract DNA and RNA for next generation sequencing. We performed targeted deep sequencing with a customized 381 cancer gene panel (CancerSCAN™) and analyzed the relationship among the sequencing data, immunohistochemistry and clinical outcome. Results: From Apr 2015 to Feb 2016, 106 patients were enrolled in the trial from 7 institutes in Korea. Of 106 patients, biomarker data were available for 79 patients. TP53 was the most frequently mutated gene (70.8%) followed by PIK3CA (45.6%). HER2 single nucleotide variant (SNV) was detected in 13 BCs (16.5%) and HER3 SNV was in 9 (11.4%). The score of HER2 immunohistochemistry (IHC) was 3+ in 68 BCs and 2+ with positive in situ hybridization in 11 BCs. In copy number variant (CNV) analysis, HER2 amplification (86.1%) was most frequently observed and followed by CDK12 amplification (58.2%) and APOBEC3B deletion (30.4%). IHC score of HER2 was positively correlated to copy number (CN) of HER2 (P=0.001) but 11 breast cancer tissue did not have copy number amplification of HER2 (13.9%) (Six of HER2 IHC score 2+ and 5 of 3+). The median progression free survival (PFS) was 4.04 months (95% CI, 2.96 - 4.40) for patients who treated with poziotinib in this study. PIK3CA activating mutations were associated with short PFS compared to wild type (WT) and other SNVs (Median PFS of activating mutations vs. WT and others: 2.66 vs. 4.40 (months), P=0.009). HER2 CN amplification was positively correlated to duration of PFS (Median PFS of no amplification vs. 4 ≤ CN < 16 vs. 16 ≤ CN: 2.56 vs. 3.02 vs. 4.86 (months), P=0.032). HER2 SNVs prolonged duration of PFS without statistical significance (Median PFS of HER2 SNVs vs. WT: 4.24 vs. 3.19 (months), P=0.114), but 10 of 13 BCs with HER2 SNV (76.9%) had clinical benefit from poziotinib and 5 BCs (38.5%) had durable response more than 6 months. Conclusion: In this biomarker analysis, SNV of HER2 was frequently observed in HER2 positive MBCs and HER2 CN amplification was detected not in all. High CN amplification of HER2 derived longer PFS than those with low CN. To contrary to this, activating PIK3CA mutations shorten PFS compared to those with WT. In addition, HER2 SNVs might be a potential biomarker of poziotinib in HER2-positive MBC. Further functional study would be warranted.
Title: Use of a functional signal profiling test with high sensitivity and specificity to determine the prevalence of abnormal HER2-driven signaling activity in the HER2-negative breast cancer patient population: New patient group may benefit from HER2 therapy

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¹Celcuity, Minneapolis, MN.

Body: Background: Biological factors, such as HER2 signaling activity, may be important to measure in addition to expression and amplification of HER2 when identifying patients eligible for HER2 therapies. The CELx HER2 Signaling Function (CELx HSF) Test measures HER2 signaling activity in live tumor cells using a label-free impedance biosensor to identify HER2-negative breast cancer patients likely to be responsive to treatment with anti-HER2 therapies. Previous studies quantified HER2-driven signaling activity in a training set (N=34) of primary tissue samples from HER2-negative breast cancer patients and found 21% of the samples had abnormal HER2 signaling. Other studies confirmed that anti-HER2 therapies, such as trastuzumab, pertuzumab, afatinib, and neratinib, are as effective in inhibiting HER2-driven signaling activity in HER2- tumor cells as they are in HER2+ tumor cells. This study set out to confirm the prevalence of abnormal HER2 signaling amongst HER2-negative breast cancer patients in a larger sample (N=114) and to characterize the sensitivity and specificity of the CELx HSF Test.

Methods: A validation set of de-identified fresh breast tumor specimens were obtained from 114 HER2- breast cancer patients. Real time live cell response to specific HER2 agonists (NRG1b or EGF) with or without an antagonist (HER2 dimerization inhibitor) was measured using an impedance biosensor. From these responses, the net amount of HER2 participation in HER2 signaling initiated by the HER2 agonists was quantified. Samples with HER2 signaling activity levels above a previously determined cut-off value were identified as abnormal.

Results: Of the HER2- breast tumor cell samples tested, 27 of 114 patients (23.7%; 95% CI=17%-32%) had abnormal HER2 signaling activity. Little or no correlation was found between a patient's HER2 signaling activity and their estrogen receptor status or tumor grade. To compare the results obtained from the training set of 34 patients and the current set of 114 patients, the Kolmogorov-Smirnov two-sample test was applied (D=0.17, P-value 0.45) and found no significant difference between the training and validation sets. A normal mixture model was fitted to the new 114 patient data set and found that HER2- breast cancer patients fall into three distinct groups (abnormal, normal, low). Patients falling into the abnormal group had mean HER2 signaling scores 4.5 standard deviations above the mean score of the normal group. A ROC curve constructed with this data projects that both the sensitivity and specificity of the CELx HSF Test would be greater than 90%.

Conclusions: These results confirm that a clinically relevant proportion of HER2- breast cancer patients, approximately 20%, have tumors with abnormal HER2-signaling activity and may benefit from HER2 therapy. With high specificity and sensitivity, the CELx HSF test may be suitable as a companion diagnostic to identify new patients eligible to receive HER2 therapies. An interventional trial to evaluate the efficacy of trastuzumab and pertuzumab in HER2- patients selected with the CELx HSF test is underway.
Title: Diffusion-weighted MRI improves imaging prediction of response in the I-SPY 2 trial

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Body: Background: The I-SPY 1 TRIAL demonstrated that functional tumor volume (FTV) measured by dynamic contrast-enhanced (DCE) MRI during neoadjuvant chemotherapy (NAC) predicts both pathologic complete response (pCR) and recurrence free survival¹. In addition to DCE, the I-SPY 2 TRIAL is testing whether diffusion weighted MRI (DWI), a non-contrast method that characterizes water mobility and cellularity by measuring the apparent diffusion coefficient (ADC), acquired during the same MRI exam as DCE, can provide valuable distinct information about tumor response. We hypothesize that combining FTV and ADC can improve the predictive performance of breast MRI.

Methods: I-SPY 2 includes women with stage II or III breast cancer with tumor size $\geq 2.5$ cm. A sub-cohort of I-SPY 2 patients from 2 graduated experimental drug arms²-³ (N=115 of 263): veliparib-carboplatin (VC, N=38), neratinib (N=37) and their controls (treated with paclitaxel or paclitaxel + trastuzumab, N=40), were included in this study: 148 patients were excluded due to missing imaging data or poor DWI quality. Each patient had four MRI exams: pre-treatment (T1), early treatment (after 3 weekly cycles of experimental drugs, T2), between regimen (T3), and pre-surgery (T4). FTV and ADC were measured for the whole tumor at T1, T2, and T3. Percent change of FTV ($\Delta$FTV) and ADC ($\Delta$ADC) at T2 and T3 compared to T1 were analyzed as predictors of pCR. The predictive performance of $\Delta$FTV, $\Delta$ADC and their combination was evaluated using a logistic regression model treating pCR as the binary outcome. Odds ratios were estimated for each 10% decrease of $\Delta$FTV and 10% increase of $\Delta$ADC to reach pCR. The likelihood ratio test was used to evaluate the effect of variables in the logistic model. The statistical significance level for all testing was set at 0.05.

Results: Out of 115 patients included in this analysis, 36 (31%) reached pCR. The combined model using $\Delta$FTV+$\Delta$ADC showed statistically significant improvement over the single predictor $\Delta$FTV alone ($p=0.038$ for the period T1 to T2 and $p<0.001$ for the period T1 to T3). The odds ratio estimates represent a 27% increase in odds for each 10% increase in $\Delta$ADC after accounting for $\Delta$FTV at T2 and 38% increase at T3 (see Table 1).

Table 1 The odds ratio of $\Delta$FTV and $\Delta$ADC with 95% confidence interval

<table>
<thead>
<tr>
<th>Treatment time point</th>
<th>$\Delta$FTV alone</th>
<th>$\Delta$ADC alone</th>
<th>$\Delta$ADC adjusted for $\Delta$FTV</th>
</tr>
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<tbody>
<tr>
<td>Early treatment (T2)</td>
<td>1.19 (1.05, 1.36)</td>
<td>1.33 (1.06, 1.70)</td>
<td>1.27 (1.01, 1.63)</td>
</tr>
<tr>
<td>Between regimen (T3)</td>
<td>1.41 (1.12, 1.98)</td>
<td>1.44 (1.24, 1.71)</td>
<td>1.38 (1.18, 1.64)</td>
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Conclusion: The addition of ADC to standard FTV MRI may help refine the prediction of treatment response. Evaluation of the method by cancer subtype in a larger cohort is ongoing.

References
Body: Triple-negative breast cancer (TNBC) is one of the most aggressive subtypes of breast cancer. TNBC affects younger women and is characterized by earlier rates of relapse, higher frequency of visceral metastases, and shorter survival outcomes when compared to ER+ or HER2+ disease. Although the disease only represents ~15% of all breast cancer cases, it accounts for 25% of all breast cancer deaths – with treatment options currently limited to chemotherapy. Development of targeted therapies for TNBC is challenging due to molecular heterogeneity and lack of high-frequency “driver” alterations amenable to therapeutic intervention. Recent studies have demonstrated increased sensitivity of TNBC to the anti-proliferative effects of Bromodomain and Extra-Terminal motif inhibitor (BETi) compared to the other breast cancer subtypes. To determine mechanisms of sensitivity to BETi, we analyzed the effect of a BETi across a panel of TNBC cell line models and identified cell lines that were both sensitive and insensitive to BETi. With the intent of identifying biomarkers of sensitivity, we performed RNA-seq and precision nuclear run-on and sequencing (PRO-seq) on both sensitive and insensitive cell line models and data generated identified significant differences in key growth regulatory and apoptotic signaling pathways, including notable differences in Myc-dependent signaling. Our data suggest potential biomarkers of BETi-sensitivity that may be of value in further pre-clinical studies. Further, our results provide mechanistic rationale for combinations of BETi with select, targeted therapies in a disease that is in need of new therapeutic intervention.
Clinical and pathologic characteristics of breast cancers determined to be HER2-positive by fluorescence in-situ hybridization (FISH) using alternative chromosome 17 probes

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Body: Background:
Based on updated 2013 ASCO/CAP guideline for HER2 testing, cases with a HER2/CEP17 ratio < 2.0 but with an average HER2 copy number ≥ 4.0 and < 6.0 signals/cell are considered equivocal. In such cases, HER2 testing using alternative chromosome 17 probes was proposed as one way to resolve the equivocal FISH results. Using the alternative probe method increases the number of cancers categorized as HER2 positive but brings to question if these cancers truly represent HER2 amplified breast cancers and derive the same benefit from anti-HER2 therapies.

Methods:
Since 2013, all breast cancers at our institution that were HER2 equivocal by traditional FISH but classified as HER2 positive using the alternative probe method were assessed for clinical and pathologic features including histologic type and grade, TNM stage, HER2: alternative probe ratio, treatment, and clinical outcome.

Results:
We identified 24 invasive breast cancers considered HER2 positive by the alternative probe method: 23 (96%) were estrogen receptor-positive (ER+) and 20 (83%) were progesterone receptor-positive. Histologically, only 2 were invasive lobular carcinomas; all others were ductal or had ductal and lobular features. Most cancers (63%) had low or intermediate histologic grade: Grade 1 (n=3); Grade 2 (n=12); Grade 3 (n=9). Clinical information was available for 18 patients: 2 had metastatic disease, 1 had a local recurrence after mastectomy and 15 patients had early stage disease; 9 with node negative disease and 6 with nodal involvement. HER2 IHC was equivocal (2+) in 16 (66.7%) cases, positive (3+) in 4 (16.7%) cases, and negative (0 or 1+) in 4 (16.7%) cases. The average HER2 copy number was 4.77, the average HER2:p53 ratio was 2.61. Repeat HER2 testing on a 2nd tumor sample was performed in 8 cases: HER2-positivity was confirmed in only 2 (25%) cases and by the alternative probe only. Treatment information was available for 17 patients: 1 had T1aN0M0 lesion and did not get chemotherapy, 16 received chemotherapy and 13 received trastuzumab-based chemotherapy. Eleven patients with early stage disease received chemotherapy and trastuzumab. Of these patients, 10/11 were ER+, 7/11 were node negative and 5/11 had grade 2 tumors, yet only one tumor was assessed by oncotype recurrence score (RS = 29). Three patients received chemotherapy and trastuzumab in the neoadjuvant setting: 1 had a complete pathologic response, 1 a partial response, and 1 has not yet gone to surgery. One additional patient received neoadjuvant chemo alone and achieved a partial response.

Conclusions:
Breast cancers considered HER2+ by the alternative probe method but not by traditional FISH are almost always ER-positive and most have low or intermediate histologic grade. Repeat HER2 testing on a subsequent tumor sample did not confirm HER2-positivity in 75% of cases. Almost all patients with early stage disease received chemotherapy and trastuzumab based on the alternative probe results without molecular assessment to predict chemotherapy response. Intrinsic molecular subtyping using PAM50 analysis on these cancers is underway to determine how many are HER2-enriched by molecular assessment.
Frequency and mechanisms of elevated somatic mutation burden in metastatic breast cancer and response to immune checkpoint blockade

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Body: Background: Immune checkpoint blockade (ICB) is effective in the treatment of various malignancies. Thus far, however, results in breast cancer have been mixed. Elevated tumor mutational load, and subsequent increased likelihood of forming immunogenic neoantigens, has been correlated with response to ICB. Mutational load observed in breast cancers varies widely. However, most studies have assessed mutational load using primary tumors. Few studies have explored the frequency of high mutational load, molecular mechanisms accounting for this phenomenon, and its potential impact on response to ICB in metastatic breast cancer (MBC).

Methods: From 2011-2016, 124 patients (pts) with MBC of varying subtypes underwent research biopsy of their metastatic disease for whole genome, exome and transcriptome sequencing of tumor and matched normal sample through the Michigan Oncology Sequencing Center (Mi-OncoSeq). Those pts with elevated somatic mutation load were defined as having greater than 10 mutations per megabase of targeted sequencing and mutational signatures accounting for high mutation load were noted. Pts treated subsequently with ICB were followed to assess response.

Results: Twelve MBC pts had high mutation load (10% of cohort). Eight pts had estrogen receptor (ER) positive MBC and 4 pts had metastatic triple negative breast cancer (TNBC). In 5 cases, a clear mutational signature accounting for high mutation load was evident. Two TNBC cases harbored an APOBEC mutational signature in addition to 1 TNBC and 2 ER positive tumors displaying a microsatellite instability signature (MSI-H). Among the tumors with MSI-H signature, 1 case was associated with a pathogenic germline alteration in MLH1. Two pts were subsequently treated with ICB on a clinical trial. One pt came off study after 3 months due to progressive brain metastases and another had partial response to therapy lasting 7 months.

Conclusions: Elevated somatic mutation burden in MBC is observed in approximately 10% of pts, and is detected in both ER positive and TNBC. Since high mutation burden has been associated with increased likelihood of response to ICB, identification of this genomic feature could have important therapeutic implications for MBC pts.
Title: Integrated analysis of PTEN protein expression and PI3KCA mutations as predictors for pathological complete response in HER2-positive breast cancer

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Body: Introduction: Anti-HER2 treatment has also influenced the response to neoadjuvant chemotherapy in HER2-positive breast cancer (BC) by increasing the pathologic complete response (pCR) rate. The activation of HER2 signaling causes the downstream activation of the PI3K/AKT/mTOR pathway that plays a crucial role in developing resistance to trastuzumab (T). Therefore, candidate biomarkers with a potential role in the prediction of a pCR and prognosis after anti-HER2 treatment could be selected from this pathway.

Material and Methods: We retrospectively evaluated PTEN protein expression and PI3KCA mutations in core biopsies from HER2-positive patients treated at our institution with neoadjuvant therapy based on anthracyclines, paclitaxel and T. Protein expression was assessed by immunohistochemistry and used as dichotomic variable, PTEN loss was considered if Hscore ≤ 40. The association of PTEN status, PI3KCA mutation and PI3K activation (defined as either PTEN loss and/or presence of PI3KCA) with pathological complete response (ypT0/isN0) and progression-free survival (PFS) was evaluated.

Results: In our database 86 patients with HER2-positive clinical stage II-III BC were identified. Median follow-up was 75.85 months. PTEN was available from 84 (97.7%) and PI3KCA genotype from 67 patients (77.9%). Median age was 47.34, stage III 58.8% and 50% were hormonal receptor (HR) positive. Low PTEN was described in 29.8% tumors and was statistically associated with Grade1-2 and HR positive tumors. PI3KCA mutations (exon 9 and 20) were observed in 23.9% tumors. PI3K activation was detected in 37.5% tumors. Overall, the pCR rate was 53.5%. In low PTEN tumors pCR was 12% and in PTEN-high tumors, it was 72.1% (p<.0001). pCR rate was statistically different between PIK3CA mutant and wild-type tumors (6.3% vs. 51.9%; p<.0001). PI3K activation was statistically associated with lower pCR rate (12.5 vs. 72.9%; p<.0001). In multivariable analysis adjusted for baseline parameters, HR expression (OR 4.170; 95% CI 0.975-17.829; p=0.054) and PI3K activation (OR 45.87; 95% CI 8.059-261.101; p<.0001) independently predicted pCR. In univariable analysis clinical, stage III, non-pCR and PI3K activation were correlated with worse PFS. PI3K activation was statistically associated with lower PFS at 5 years (OR 3.813; CI 95% 1.369-10.347; p=0.009) in the multivariable analysis.

Conclusions: The study showed the potential role of PIK3CA Genotype and PTEN expression in predicting pCR and prognosis after anthracycline-taxane-based chemotherapy and anti-HER2 treatment.
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Title: New quantitative diagnostic method by fluorescence nanoparticle for HER2 positive breast cancer treated with neoadjuvant lapatinib and trastuzumab: The Neo LaTH study (JBCRG-16TR)

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Body: Background: HER2 (human epidermal growth factor receptor 2) testing performed by IHC (immunohistochemical) methods and FISH (fluorescence in situ hybridization) is semi-quantitative. Exact quantification of HER2 is needed to predict which patients are more or less likely to respond to anti HER2 therapy. To improve the method for cancer patients' HER2 status, we developed a novel fluorescence IHC method using new fluorescence nanoparticle. The fluorescent intensity of this new nanoparticles, termed phosphor-integrated dot (PID), was approximately 100-fold brighter than that of Quantum dots. Because of its increased brightness and analyzing technology, this PID-based fluorescent IHC(IHC-PIC) has an ability of quantifying the biomarker protein in the cancer tissue sample at single particle level. In this study, the primary objective was to investigate if pathological complete response (pCR) rate in HER2- positive breast cancer treated by trastuzumab and lapatinib containing neoadjuvant systemic therapy would depend on the level of HER2, EGFR, HER3, Ki67, ER and PgR protein quantified by this new method.

Methods: The Neo-LaTH study is a randomized phase II multicenter trial evaluating the efficacy and safety of lapatinib and trastuzumab followed by lapatinib and trastuzumab plus weekly paclitaxel with or without prolongation of anti-HER2 therapy prior to chemotherapy (18 weeks vs. 6 weeks). The primary endpoint was the comprehensive pCR rate. We evaluated the HER2, EGFR, HER3, Ki67, ER and PgR amount by nano-patho method using PID in formalin-fixed paraffin-embedded core biopsy samples taken at diagnosis retrospective analysis. Univariate and multivariate analyses were performed to determine the association between pCR and variables, including HER2, EGFR, HER3, Ki67, ER and PgR nano-patho score and clinicopathological factors including histological grade, tumor status, nodal status and HER2 FISH ratio.

Results: A total of 96 tumor samples from patients were used for the present analysis. The pCR rate was 60.4%. We obtained the images of only PID signal by the image analyses, and calculated the number of PID particles in a cell and defined it as IHC-PID score that reflects the level of HER2, EGFR, HER3, Ki67, ER and PgR protein expression in cancer cells. Univariate analysis showed that HER2 IHC-PID score(p<0.0001), ER IHC-PID score(p=0.009) and PgR IHC-PID score(p=0.019) were associated with pCR and multivariate analysis showed that HER2 IHC-PID score was significantly associated with pCR (adjusted odds ratio, 0.990 [95% CI, 0.984–0.996]; P < .0001).

Conclusion: We successfully performed the quantitative IHC-PID for HER2, EGFR, HER3, Ki67, ER and PgR. And we propose using HER2 IHC-PID score as a predictive factor for trastuzumab and lapatinib containing neoadjuvant systemic therapy. This quantitative diagnostic method would be expected to contribute to the development of a molecular therapeutic strategy.
Potential recurrence markers of locally advanced triple negative breast cancer treated by combined neoadjuvant EGFR targeting and chemotherapy, revealed by genomic analyses and assessment of tumor-infiltrating lymphocytes

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Body: Background: Epidermal growth factor receptor (EGFR) is expressed in ~50% of triple negative breast cancer (TNBC) and has been proposed as a therapeutic target in this disease. However, trials testing EGFR blockade in TNBC failed to show significant clinical benefit. Probable reasons for such results were patient selection based on EGFR expression and the enrollment of heavily pretreated metastatic patients. Our team has conducted two neoadjuvant trials testing the activity of the anti-EGFR antibodies panitumumab (PTB) and cetuximab (CTX) combined with chemotherapy in locally advanced TNBC. Biomarkers predictive of pathological complete response (pCR) were the level of tumor cell EGFR protein expression and tumor-infiltrating lymphocytes' (TILs) profile (PMIDs 24827135, 26649807). The PTB-treated pts had a higher pCR rate (47%) than the CTX-treated pts (24%), but also a twice higher relapse rate, after 5 years of follow-up. Here we report results of genomic and TILs studies, performed in order to reveal possible determinants of recurrences in those trials. Methods: Tumor tissues sampled before and after neoadjuvant therapy (NAT) have been analyzed by next generation sequencing (NGS) using a targeted exome panel (MSK-IMPACT) of 410 cancer-related genes. Gene expression was evaluated by Affymetrix arrays. TIL density was assessed on pre-NAT samples according to Salgado et al, 2014 (PMID 25214542). The correlation between response, recurrences, genomic and TIL findings was analyzed in a case-by-case fashion. Results: Sixteen tumors that achieved pCR (PTB: 11, CTX: 5) and 23 non-pCR tumors (PTB: 11, CTX: 12) have been analyzed. For 14 non-pCR tumors (PTB: 6, CTX: 8) data have been obtained both from the pre-NAT and the post-NAT sample. Among those tumors, 6 recurred within 2 years after surgery (PTMB: 3, CTX: 3) and assays are on-going on several others that relapsed. Several genomic aberrations that potentially played a causative role in opposing to therapy were identified. We observed multiple mutations in the PI3K pathway in several non-pCR or relapsing pts. Interestingly, in a residual tumor (RT) of a non-pCR patient we found 3 different activating mutations in PIK3CA and one in PTEN. Another example of genomic selection induced by pharmacological pressure is the emergence of a HRAS G12S mutation in a RT after CTX. Additional novel findings include in-frame mutations and deletions in ARID1B and PARP1 amplification in non-pCR pts. Most of the tumors which recurred had ≤10% TILs (9/13) and only 4/13 had ≥30%. Among the tumors with a post-NAT RT but without recurrence, 17/33 had ≤10% TILs and 16/33 ≥30%. No particular link between TIL density and mutation pattern was observed. Conclusions: This is an example of a case-by-case approach where we captured the intrinsic inter-tumor heterogeneity, which is likely responsible for the different responses to EGFR-targeting in TNBC. Genes/pathways candidate of resistance to therapy are currently being validated.
Title: A gene expression signature that predicts for trastuzumab response in HER2+ breast cancer

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Background: Approximately 15-20% of breast cancers overexpress HER2. These patients are eligible for trastuzumab in combination with chemotherapy. However, some patients are extreme responders to single agent trastuzumab and we wanted to identify differences in cancer gene expression that could predict response to single agent trastuzumab.

Methods: We performed paired-end RNAseq on an isogenic cellular model of trastuzumab sensitivity and resistance. We reasoned that the isogenic nature of the cellular clones used in this study would enrich for differentially-expressed genes (DEGs) that were associated with response to single-agent trastuzumab. DEGs where chosen based upon either i) large fold changes in resistant vs. sensitive clones, ii) high frequency in human HER2+ breast cancers, or iii) were found to be enriched with other DEGs in signaling pathways selected by Ingenuity Pathway Analysis (IPA). DEGs were further scrutinized based upon associations with overall survival (OS) in HER2+ human breast cancers. The resulting genes were validated using qPCR and in several independent sample sets containing gene expression profiles of human breast cancers.

Results: Discovery: RNAseq yielded 3,241 statistically-significant DEGs. We used two independent filtering pipelines to obtain 175 DEGs. Ingenuity Pathway Analysis found signaling pathways associated with eukaryotic initiation factor, lysine specific demethylase 5B, and estrogen receptor alpha to be enriched in DEGs associated with trastuzumab resistance. Of these DEGs, six genes correlated with a statistically significant change in OS in the training dataset, and were validated by qPCR in the cell lines used for the analysis. We further determined that the six-gene signature was a negative predictor of overall survival in HER2+ breast cancer patients whose cancers carried at least one DEG. Validation: Using independent cohorts from TCGA and the website KMplot.com, we validated the predictive power of the six-gene signature. Of the 47 HER2+ patients from TCGA, eight patients carried two more DEGs, while 39 carried ≤ 1 DEG. Although the numbers are small, of the 8 patients followed for four or more years, only one patient was alive as compared with 7 out of 39 patients without the signature. Similarly, Kaplan Meier analysis of gene expression data from KMplot.com revealed that only 1 out of 23 patients (4.3%) who carried high mean expression of the six-gene signature were free of distant metastases after 87 months, compared to 4 out of 43 patients (9.3%) from the cohort carrying low mean expression of the six-gene signature. In both validation cohorts, the six DEG signature was not predictive in HER2-negative breast cancers.

Discussion: Patients whose tumors lack this gene expression signature are more likely to experience a favorable response to trastuzumab therapy. This signature requires validation in a clinical cohort treated with trastuzumab monotherapy.
Title: Predictive impact of absolute lymphocyte counts for progression-free survival in HER2-positive advanced breast cancer treated with pertuzumab and trastuzumab plus eribulin or nab-paclitaxel

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Body: Background: Tumor-infiltrating lymphocytes might be a one of predictive outcome of human epidermal growth factor receptor 2 (HER2)-positive advanced breast cancer (ABC) patients (pts) who treated with trastuzumab and pertuzumab (TP) plus docetaxel. Although peripheral blood-based parameter (PBBP) is reported as a prognostic indicator of patients with early breast cancers, utility of PBBP has not been studied in HER2-positive ABC.

Objective: The aim of our study was to determine whether PBBP is significant for predictive efficacy in HER2-positive ABC treated with TP combined with eribulin (ERI) or nab-paclitaxel (Nab-PTX).

Methods: The 51 patients' data from two single arm phase II trials was included in this retrospective-prospective study; ERI + TP (n=30) or Nab-PTX + TP (n=21) registered with UMIN000012375 or UMIN000006838, respectively. We assessed the PBBP in prospectively collected data and investigated their association with progression-free survival (PFS). In consideration of PBBP, we evaluated absolute lymphocyte count (ALC), neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR). The cutoff values of ALC, NLR, and PLR were set at 1000 cells/µL, 2, and 250, respectively.

Results: Median age at baseline was 58 years (range: 31-77). Median number of previous chemotherapy was 3 (range: 1-10). Pts had multiple metastases, 53% with LNs, 35% with bone, 25% with lung, 20% with liver, and 6% with brain. The objective response rate (CR+PR) and clinical benefit rate (CR+PR+ more than 6 month SD) were 37% (n=19) and 59% (n=30), respectively. The median PFS of all pts was 301 days (range: 21-1281). The PFS of pts with ALC-High was significantly better than those of ALC-low (hazard ratio (HR): 2.74, 95% confidence interval (CI): 1.28 to 5.86; p= .0097). Furthermore, improved PFS was obtained in pts with ALC greater than 1500 cells/µL compared with less than 1000 cells/uL (HR: 4.05, 95% CI: 1.60 to 11.6; p= .0029). Significant associations seem to exist irrespective of number of previous chemotherapy. Since we combined different studies for evaluating PBBP, ERI and Nab-PTX were calculated separately. Marginally significant associations between ALC and PFS were obtained both in ERI (HR: 2.18, 95% CI: 0.87 to 5.60; p=.0973) and Nab-PTX (HR: 3.26, 95% CI: 0.80 to 12.4; p=.0939). The PFS of NLR-low pts was significantly better than those of NLR-high (HR: 2.29, 95% CI: 1.01 to 5.90; p=.0477), but this statistical difference was inferior to those of ALC. There was no significant association between PLR and PFS.

Conclusions: Pre-treatment ALC-High was significantly correlated with favorable PFS of pts treated with TP irrespective of combination chemotherapy in HER2-positive ABC. Prolonged PFS of TP combination therapy might be obtained mediating through host systemic onco-immunity. These data obtained here suggest that a usefulness of ALC for selecting pts who might have clinical benefit from TP combination therapy for heavily treated HER2-positive ABC.
Title: Ki-67 index value and progesterone receptor status predict prognosis and suitable treatment in node-negative breast cancer patients with estrogen receptor positive and HER2 negative tumors

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Body: Background: Breast cancer is no longer a single disease with high molecular heterogeneity. Gene profiling has identified at least 4 subtypes: Luminal A, Luminal B, HER2-enriched and basal-like breast cancer. Moreover, immunohistochemistry (IHC) classification is now considered a surrogate for establishing breast cancer subtypes. In previous report Luminal A was defined as ER and PgR positive, HER2 negative, Ki-67 low and recurrence risk low based on the multi-gene-expression assay. The distinction between Luminal A-like and Luminal B-like can be made by either using a high Ki-67 value (≥20%) or a low PgR value (< 20%). In this study, patients with ER positive, HER2 negative and negative node were classified into 4 groups according to the PgR and the Ki-67 status (cutoff points: 20%) and examined retrospectively in relation to clinicopathological findings including the recurrence score (RS) and disease-free survival (DFS).

Methods: A total of 1866 invasive breast cancer patients from November 2001 to November 2016 were included in this study. The cases were classified as follows; LA as high PgR/low Ki-67 (850 cases), LB1 as high PgR/high Ki-67 (553 cases), LB2 as low PgR/high Ki-67 (226 cases), and LB3 as low PgR/low Ki-67 (237 cases). Out of all these cases, 1510 were treated with endocrine therapy alone. The median follow-up period was 78.1 months. Moreover, 23 of the cases underwent a 21-gene expression assay and the RS (< 25 and > 26) was compared with our classification.

Results: The median age was 57.4 years (range: 25 - 94). T1 tumors were more common in the LA group and rare in the LB2 group. Nuclear grade 3 and p53 overexpression were significantly correlated with LB2. Endocrine therapy alone was performed in 87.4% (LA), 77.4% (LB1), 58.8% (LB2) and 86.9% (LB3), retrospectively. There were significant differences in DFS between the LA group (5y DFS: 98%, 10 y DFS: 95.9%) and the LB2 group (5y: 89.9%, 10y: 83.6%; p<0.0001) or LB1 (5y: 94.9%, 10y: 89.5%; p<0.0001), but there was no difference with the LB3 group (5y: 98.6%, 10y: 94.7%; p=0.88). In the cases with endocrine therapy alone, LA showed a similar DFS with LB3 (p=0.25). LB2 had a significantly worse DFS in all the cases and in the cases with endocrine therapy. Chemotherapy was administered to cases with a higher nuclear grade in combination with endocrine therapy. In the LB2 group, there was no difference in DFS between the cases with endocrine therapy and in the cases with chemo-endocrine therapy. Moreover, most of the cases with LA (1/1) and LB1 (15/16) had a RS of <25, and all of the LB2 (6/6) cases had a RS of >26.

Conclusion: The patients with LA and LB3 (both: Ki-67<20%) had a favorable DFS even in the endocrine therapy alone group. However, LB1 and LB2 (both: Ki-67≥20%) had a poorer DFS. Moreover, LB2 (PgR<20% and Ki-67≥20%) was significantly correlated with a higher degree of malignancy and benefited from chemotherapy. LA and LB3 with low Ki-67 values were considered to be a part of the Luminal A group. These data suggest that PgR and the Ki-67 status are useful in predicting prognosis and deciding the treatment strategy for patients with ER-positive and HER2 negative breast cancer.
Title: Tumor-infiltrating lymphocytes and PD-L1 expression in HER2-positive breast cancer treated with neoadjuvant chemotherapy

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Body: Backgrounds: Neoadjuvant chemotherapy is one of the standard therapeutic strategies for HER2-positive early breast cancer. Tumor-infiltrating lymphocytes (TILs) have been shown to predict response to the neoadjuvant chemotherapy. Programmed death 1 ligand 1 (PD-L1) is a transmembrane protein, which is an immune regulatory molecule that limits antitumor immune activity. PD-L1 is expressed in a variety of cancers including breast cancer and its expression has been correlated to TIL. In this study, the relationships between TIL and PD-L1 expression and the efficacy of the neoadjuvant chemotherapy (NAC) in HER2-positive primary breast cancer were investigated. Patients and Method: Seventy-five patients with HER2-positive breast cancer at clinical stage I-IIIB, who were treated with NAC, were included in this study. Anthracycin (A) and taxane (T) (A/T) were used for 33, A and T+anti-HER2 therapy (A/T+anti-HER2) were used for 42 patients. TILs and PD-L1 was evaluated using the sections obtained by core needle biopsy before NAC. TILs were evaluated on hematoxylin and eosin (H&E)- stained sections, and reported for the stromal compartment within the borders of the invasive tumor. PD-L1 expression was analyzed by immunohistochemistry and the immunoreaction was evaluated in the tumor cells in the invasive area.

Results: Pathological complete response (pCR) was obtained in 33 patients (44%). The pCR rate was significantly higher in the patients treated with A/T+anti-HER2 than those treated with A/T alone (p=0.0080). The prognosis of the patients who achieved pCR was significantly better than that of those without pCR, in terms of recurrence-free survival (p=0.0464) and distant-metastasis-free survival (p=0.0447). TILs were < 10% in 17 (22.7%), 10-50% in 44 (58.7%) and ≥ 50% in 14 (18.7%) patients. PD-L1 was positive in 21 (28%) patients. PD-L1 expression was positively correlated with TIL (p=0.0044). High TIL was significantly associated with high pCR rate (p=0.0014); however, PD-L1 positivity itself was not associated with pCR rate. High TIL was significantly associated with high pCR rate, especially in A/T+anti-HER2 group. Intriguingly, PD-L1 expression seems to have negative impact on pCR even in the same range of TILs. Tumors with high TILs and the negative PD-L1 showed the highest pCR rate.

Conclusion: High TIL and negative PD-L1 expression in the tumor cells was significantly associated with high pCR rate, especially in the patients treated with anti-HER2 therapy.
Publication Number: P2-09-34

Title: An mRNA-based method to measure PI3K activity in cancer tissue using a computational pathway model to assess FOXO transcriptional activity

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Body: Introduction
The PI3K signaling pathway is frequently active in breast cancer, and therapeutic inhibitors have been developed. However, it has proven difficult to correctly predict treatment response. We developed a method that measures functional activity of the PI3K pathway using a computational model that infers transcriptional FOXO activity (downstream of PI3K) from expression levels of its target genes. In principle, PI3K pathway activity inhibits transcriptional FOXO activity, hence inactive FOXO is indicative of active PI3K.

Method
We developed a knowledge-based computational model to infer transcriptional FOXO activity from cancer tissue mRNA expression levels, using a Bayesian network approach (Verhaegh et al., Cancer Res 2014). Model calibration was done on microarray data from HUVEC cells with inducible FOXO3.A3-ER (GSE16573).

Results
The FOXO model was biologically validated with in-house microarray data from independent breast cancer cell lines. ER positive, PIK3CAEs54K mutant MCF7 and triple negative MDA-MB-231 cells were stably transduced with a doxycycline inducible FOXO3.A3 expression vector, allowing controlled induction of FOXO3 protein activity. FOXO activity was determined to be low in untreated and 20% FBS treated MCF7 cells, and high after doxycycline, LY294002, and combination treatment. Next, we tested our FOXO model on independent MCF7, BT-20 and MDA-MB-453 cell line data treated with EGFR inhibitor erlotinib (GSE30516), showing an increase of FOXO activity upon treatment, due to reduced PI3K pathway activity (combined Wilcox rank sum test p = 7.8x10⁻⁵).

We further analyzed independent publicly available data from breast cancer patients. FOXO was generally active in healthy breast tissue. Compared to healthy breast tissue, FOXO activity was higher in normal-like and luminal A breast cancer samples (p = 1.9x10⁻⁶ and 0.025, resp.), and lower in luminal B samples (p = 4.2x10⁻⁷).

In addition to the above mechanism for regulating FOXO activity, literature suggests that FOXO can also be activated by cellular oxidative stress, which is often associated with PI3K signaling. This may be assessed using expression levels of the FOXO target gene SOD2, which is differentially expressed between the two FOXO activity modes, and whose function is to reduce oxidative stress. Public data shows an increasing percentage of elevated SOD2 levels among FOXO-active samples with increasing breast cancer aggressiveness: 7% in normal-like, 5% in luminal A, 18% in luminal B, 31% in HER2-enriched and 74% in basal like breast cancer.

Conclusion
Our computational model to measure PI3K activity using FOXO target gene mRNA levels was able to measure increased FOXO activity in multiple cancer cell lines after PI3K inhibition. FOXO activity was measured high in healthy breast tissue and in normal-like and luminal A breast cancer, and lower in luminal B, indicating PI3K activity in the latter group. In more aggressive subtypes, FOXO activity was increasingly accompanied by high SOD2 expression, suggesting oxidative stress with associated PI3K activity as the FOXO activating mechanism.

Clinical utility for improved response prediction and monitoring of PI3K pathway inhibitors is being investigated with clinical partners.
Title: Validation that a histone gene signature predicts anthracycline response in early breast cancer

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Body: Background: The use of anthracycline-based chemotherapies has improved overall and disease free survival in breast cancer. However, anthracyclines can have significant toxicities including cardiotoxicity and leukemia. It is, therefore, imperative to identify those patients who will benefit from adjuvant anthracycline treatment and patients who could be spared unnecessary toxicities and be considered for alternative adjuvant therapy. Previous work performed by our laboratory identified a histone gene expression signature as a predictive marker of anthracycline benefit in the BR9601 clinical trial. In this study we validate the 18 histone gene signature in the MA.5 clinical trial and examine the role of the signature in individual intrinsic subtypes of breast cancer.

Methods We analysed the CCTG MA.5 clinical trial in a prospectively planned retrospective biomarker approach to validate this signature and tested the role of intrinsic subtyping as predictive markers of anthracycline benefit. RNA was extracted from patients in the MA.5 adjuvant trial evaluating the addition of epirubicin (E) to CMF and analysed using NanoString technology. Log-rank analyses validated the predictive values of the signature on distant relapse-free survival (DRFS). Cox-regression models tested independent predictive value on DRFS in the presence of treatment, age, tumour size, nodal status, HER2, ER status and grade, and treatment by marker interactions.

Results Analysis of the MA.5 clinical cohort revealed that patients whose tumour had low histone gene signature expression experienced increased DRFS (HR: 0.54, 95% CI 0.38-0.76, p=0.001) when treated with CEF compared with patients treated with CMF alone. Conversely, there was no apparent benefit of CEF vs CMF in patients with high histone gene expression signature (HR: 1.01, 95%CI 0.66-1.55, p=0.963). After multivariate analysis and adjustment for HER2, nodal status, age, grade and ER, the treatment by marker interaction for the gene signature was 0.54 (95%CI 0.31-0.94, p=0.030) for DRFS. The predictive impact of the histone signature was independent of intrinsic subtype.

Conclusion The histone gene expression signature is an independent predictor of anthracycline benefit and could be a potential candidate diagnostic assay for patients with early breast cancer.
Title: Basal biomarkers nestin and INPP4b predict gemcitabine benefit in metastatic breast cancer: Results from the phase III SBG0102 clinical trial

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Body: Background: A growing body of evidence is suggesting that basal-like and triple negative breast cancers may be particularly sensitive to nucleoside analogues (gemcitabine, capecitabine). In a prospective-retrospective analysis of the phase III SBG0102 clinical trial randomizing metastatic breast cancer patients to gemcitabine plus docetaxel (GD) or to higher-dose single agent docetaxel (D), patients with basal-like breast cancer by PAM50 gene expression had significantly better overall survival (OS) in the gemcitabine arm. By immunohistochemistry (IHC), triple negative status was not predictive, but is a poor surrogate for the basal-like intrinsic subtype. More accurate IHC biomarkers have since become available defining basal-like breast cancers by nestin positivity or by loss of inositol polyphosphate-4-phosphate (INPP4b).

Methods: Formalin-fixed paraffin embedded blocks of primary tumor tissue corresponding to 270 of the 337 patients participated in the SBG0102 trial were used to build tissue microarrays. IHC staining and interpretation for nestin and INPP4b by pathologists (who had no access to clinical data) followed published methods. A prespecified statistical plan was executed independently by Danish Breast Cancer Group statisticians, testing the primary hypothesis that patients with basal breast cancer – defined as positive for nestin or negative for INPP4b, regardless of ER/PR/HER2 status – would have superior OS on the GD treatment arm when compared to the D treatment arm by interaction test. Secondary outcomes included time to tumor progression (TTP) and response rate. Kaplan-Meier method with log-rank test of nestin and INPP4b status was used to measure OS and TTP. Forest plots were used to visualize predictive capacities relative to IHC markers and treatment effects.

Results: Two hundred fifty two cases were evaluable for this study, among which 38 (15%) had been classified as basal-like, 45 (18%) as HER2-Enriched, 74 (29%) as luminal A and 91 (36%) as luminal B by PAM50. Among 241 cases being evaluable for both IHC nestin and INPP4b markers, positive staining of nestin or loss of INPP4b was observed in 43 (17%) of the total cases and was significantly associated with PAM50 basal-like subtype (p<0.0001). Within a median follow up of 13 years, patients assigned as IHC-basal by virtue of being “nestin+ or INPP4b-” demonstrated a significantly lower OS when compared to non-basal cases defined as “nestin- and INPP4b+” (HR=2.45, 95% CI: 1.47-4.07) (p=0.0006). The IHC-basal patients did much better on the GD vs. the D arm (HR=0.36, 95% CI: 0.19-0.68) whereas there was no such difference in outcomes for other patients (HR=0.99). The interaction test was significant (p-interaction<0.005).

Conclusions: The nestin/INPP4b IHC panel offers a practical and inexpensive technology to identify basal-like patients. In the metastatic setting, women with IHC-basal breast cancers defined using these markers have superior overall survival when randomized to gemcitabine-containing chemotherapy compared to docetaxel alone.
Title: Genomic markers but not molecular subtypes provide prognostic impact and predict anthracycline efficacy in early triple-negative breast cancer: Results from the prospective WSG PlanB trial

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Body: Background: Optimal treatment, particularly use of anthracyclines in aggressive triple-negative breast cancer (TNBC), is still a controversial issue in early BC management. However, TNBC exhibits substantial molecular heterogeneity: for example, the immune phenotype seems to be associated with better outcome. An important clinical issue in early TNBC is to quantify the impact of subtypes as well as individual genes on survival and especially on anthracycline benefit.

Methods: In PlanB, patients with ER and PR<1% (local or central lab), HER2- EBC were treated by TC (6 cycles Docetaxel/Cyclophosphamide) or EC-Doc (4xEpirubicin/Cyclophosphamideà4xDocetaxel) (overall n=2449, HER2-). RNA isolation was successfully performed in n=402/449 patients with available follow-up. Gene (n=119) expression data by Nanostring\textregistered platform were entered into univariate and multivariate Cox models for disease-free survival (DFS) to identify genes (and combinations) with potential prognostic and/or predictive impact. Median follow-up was 60 months.

Results: RNA expression results were available in n=394 (203 TC vs. 191 EC-Doc): PAM-50 subtype: basal-like 82%; HER2-enriched 7%; luminal (A or B) 3.5%; normal-like 7.4%. Median age was 54; 78% were node-negative. In patients with “discordant” tumors (HR positive by local or central assessment), 76% were still basal-like, compared to 86% in “concordant” TNBC. Of 27 patients with HER2-enriched subtype, HER2 status was positive by central assessment in only five cases (18%). Within this TN cohort, 5y DFS was similar in TC (83%) and EC-Doc (79%) arms; positive nodal status and tumor size >2 cm were (unfavorable) clinical-pathological prognostic markers. Prognostic or predictive impacts of molecular subtype, risk of recurrence subgroups, or proliferation indices were not seen.

Twelve genes (incl. CD8, EGFR, GPR160, SPINT2) showed potential multivariate prognostic impact by entering the “forwards stepwise” multivariate Cox model for DFS. The upper half of patients according to the resulting “twelve-gene signature” had well over 90% 5y-DFS, whereas the lowest quartile had under 60% 5-y DFS. Several genes (incl. ERBB2, FOXC1) showed potential for a predictive impact regarding TC vs. EC-Doc by interaction analysis. Further details and perspectives for testing the robustness of these potential impacts will be presented at the meeting.

Conclusions
To our knowledge, these are the first results from a prospective, adjuvant taxane-based trial regarding molecular predictors of anthracycline efficacy and PAM-50-based prognostic factors in early TNBC. ERBB2 expression, but not HER2-enriched subtype, was predictive for A-benefit in HER2-negative BC. Molecular heterogeneity of TNBC beyond basal-like vs. non-basal-like subtype is clinically relevant and should be considered for patient stratification in ongoing trials with combination therapy. The identified prognostic gene signature should be validated in the WSG-ADAPT-TN and other TNBC trials.
Targeted sequencing in early breast cancer: Identification of novel candidate mutations predictive of anthracycline benefit

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Body: Background The use of chemotherapies such as anthracyclines and taxanes have improved overall and disease free survival in breast cancer. For all patients, anthracyclines can have significant toxicities including cardiotoxicity and leukemia. It is therefore essential to select the subset of patients who will receive the optimal overall benefit from anthracycline therapy and to identify molecular pathways driving resistance. To fully understand the impact of mutations in the context of current breast cancer therapy, requires a comprehensive mapping of key molecular events in the context of treatment. We sequenced 101 genes, that were prioritized based on not only gene frequency, but also taking into account the importance of amino acid substitution, type of mutation and network connectivity, in 692 primary tumours to both identify driver genes and pathway cassettes and to understand their clinical significance in response to anthracycline treatment.

Methods We performed targeted sequencing in patients from the BR9601 (n=374) and CCTG MA.5 (n=703) clinical trials. The BR9601 and MA.5 clinical trials examined the effectiveness of combination chemotherapy consisting of CMF (cyclophosphamide, methotrexate and 5-fluorouracil) with or without epirubicin. DNA was extracted, samples were sequenced using AmpliSeq Technology adapted to Illumina and somatic mutations were called using a novel mutation calling pipeline (ISOWN). A priori analyses were performed using distant recurrence free survival (DRFS) as the primary endpoint.

Results: In 692 successfully analysed samples 509 (73.6%) samples exhibited at least one single nucleotide mutation (range 0-54). 94/101 genes were mutated in at least one patient. Only variants in PIK3CA, TP53, CDH1, TLE6, MLL3 and USH2A were detected in 5% or more of samples. TSC22D1, RB1 and ZNF565 were associated with increased risk of distant relapse in multivariate analyses corrected for clinic-pathological variables. No single genes were predictive of anthracycline treatment compared to CMF in multivariate analyses corrected for clinic-pathological variables. Signaling cassettes/modules were designed based on the pathway database, Reactome. Within the signaling cassettes one module was predictive of anthracycline failure. Patients with one or more mutations in this module had an increased risk of distant relapse (HR 0.52, 95% CI 0.29-0.95, p=0.034) when treated with an anthracycline containing chemotherapy regimen compared to CMF (HR 1.34 95% CI 1.05-1.72, p=0.019).

Conclusions: We successfully performed a signaling pathway-based targeted sequencing analysis within predefined signaling modules. We identified a single signaling cassette linked to anthracycline resistance in early breast cancer. However, further work to validate this study in a separate clinical trial is warranted.
Title: The impact of TP53 mutation and pathological response to neoadjuvant chemotherapy on triple negative breast cancer outcomes

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Body: Background:
Triple Negative Breast Cancer (TNBC) accounts for 15-20% of breast cancers, and has a poor prognosis compared with hormone receptor-positive or HER2-amplified disease. There is currently no targeted therapy to salvage chemotherapy resistant TNBC confirming a need for predictive biomarkers of sensitivity to therapy. This study aimed to evaluate the impact of TP53 mutations, common in TNBC, on sensitivity to therapy and patient outcomes.

Methods:
TNBC samples (pretreatment biopsies and surgical specimens, n=106) had annotated response to standardized anthracycline/taxane based neoadjuvant chemotherapy (NACT) and 3-year survival data, by which time most recurrences occur in TNBC. RNAseq was performed using Illumina HiSeq with mRNA library preparation protocol to identify TP53 mutations, which were then scored using the Evolutionary Action Score (EAS), a novel computational model shown to be successful at identifying deleterious mutations. This work was supported by an ASCO Conquer Cancer Foundation Young Investigator Award grant.

Results:
106 TNBC patients were enrolled on this study, 14 had stage I, 65 stage II, and 24 had stage III disease. 64 patient samples have been analyzed to date, of which 39 (61%) harbored a TP53 mutation, and 24 (38%) were TP53 wildtype.

Pathologic complete response (pCR) and survival
Of 47 patients who received NACT, 14 (30%) achieved a pCR, and 33 (70%) had residual disease (RD). Patients who achieved a pCR had a significantly better 3-year recurrence free survival (RFS) (100% vs 59%, p=0.011) and overall survival (OS) (100% vs 62%, p=0.01) compared to patients who had residual disease.

TP53 status and pCR
The rate of pCR was significantly higher in the TP53 mutant group (11/29, 38%) compared to TP53 wildtype (2/17, 12%) TNBC (OR = 4.6, (0.9, 24), p = 0.046). Patients with wild-type and mutant TP53 TNBC who achieved a pCR had excellent 3-year RFS and OS at 100%. Among patients with RD, TP53 wild-type TNBC exhibited a non-significant trend towards higher 3-year RFS (72% vs 49%,HR = 2.5 (0.8, 8.0) p = 0.12) and OS (79% vs 48%,HR = 2.6 (0.8, 8.4) p = 0.11) compared to TP53 mutant TNBC.

TP53 status and survival
The 3-year OS for TP53 mutant TNBC was 72%, compared to 86% for TP53 wildtype TNBC (HR = 1.5 (0.5, 4.3), p = 0.48). There was no difference in 3-year RFS between the two groups.

TP53 EAS and survival
Among 39 non-silent TP53 mutant cases, EAS ranged from 42.3 to 100 with a median score of 87.6. The 3-year OS for patients with EAS ≥66.7 was 73%, compared to 74% for patients with EAS <66.7 (HR 0.8 (0.2, 3.0), p=0.78). When separated into tertiles, the 3-year OS rates were 75%, 68%, and 77% for the lower, middle, and upper thirds respectively. The 3-year OS rates for TNBC with EAS = 0 (TP53 wildtype or silent TP53 mutation) was 87%.

Conclusion:
TP53 mutations were associated with a higher chance of achieving a pCR; NACT Patients who achieved a pCR to NACT had excellent outcomes, regardless of TP53 mutation status. In patients with RD following NACT, TP53 mutant TNBC had a trend towards worse outcomes compared to TP53 wild-type TNBC. Larger cohorts are required to further evaluate the impact of TP53 mutations and TP53 EAS on survival outcomes in TNBC.
Title: Identifying patients sensitive to anthracycline-containing therapy with quantitative proteomic and genomic profiling

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Body: Background: Selecting chemotherapy based on tumor biology can improve response rates and avert toxicity. Studies of the relationship between tumor expression of TOP2A protein and response to anthracycline-based chemotherapy have yielded contradictory results. Here we used mass spectrometry (MS) to evaluate associations between tumor molecular profiles and pathological complete response (pCR) in breast cancer patients treated with neoadjuvant anthracycline-containing therapy.

Methods: Patients were selected from the ERNEST-B (Erlangen Neoadjuvant Study Breast), which is a retrospective cohort study. Archived tumor samples from anthracycline-treated breast cancer patients (n=133) were microdissected and solubilized. In each tumor sample, TOP2A and other target proteins were quantitated with a mass spectrometry assay. Molecular profiling also included RNA sequencing of the tumor and whole genome sequencing of both tumor and matched normal tissue sections. The cohort was dichotomized into high and low expressors of TOP2A using a protein level cutoff of 515 amol/ug of tumor protein. The difference in pCR (ypT0ypN0) rates between high and low expressors of TOP2A was assessed using a z-test for differences in proportion.

Results: TOP2A protein was detected in 84 of 133 (63%) tumor samples from anthracycline-treated patients (range: 178 to 3044 amol/ug). Patients whose tumor expressed TOP2A protein above the cutoff of 515 amol/ug had higher pCR rates than patients with lower TOP2A expression (35.5% vs. 12.1%; odds ratio (OR): 4.48 [95% CI: 1.53-13.28], Fisher’ exact test p = 0.004). The difference retained statistical significance in a logistic regression model adjusting for HR and HER2 status (OR: 2.23 [95% CI: 1.15-4.30], p=0.017). In a separate cohort of 19 breast cancer patients who did not receive anthracycline, there was no association between TOP2A protein expression level and pCR rate. Results from a validation cohort as well as the genomic analysis will be presented at the meeting.

Conclusions: In a retrospective analysis of anthracycline-treated breast tumors, TOP2A expression was associated with a higher rate of pCR. Targeted proteomics may predict the response of breast cancer patients to anthracycline-based therapy.
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Publication Number: P2-10-07

Title: A prospective clinical utility study of the impact of the 21-gene recurrence score (RS) assay in the treatment of estrogen receptor positive (ER+), HER2 negative (HER2-), 1-3 node positive (N+) breast cancer (BC)

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Body: Background:
The 21-gene RS is routinely used in node negative ER+, HER2- BC to help guide chemotherapy treatment decision making. There is emerging use of the RS in limited N+ BC, though the results from the randomized clinical trial (RxPONDER) is pending. The objective of the study is to determine whether the results of the 21-gene RS will alter physician treatment decisions in ER+ HER2- limited N+ BC.

Methods:
Consenting patients (pts) with ER+, HER2-, 1-3 N+ (microscopic or macroscopic) early stage BC participated in this prospective, multi-center British Columbia Cancer Agency (BCCA) study. Treating physicians completed a questionnaire after an initial visit with the patient to document their treatment recommendation before obtaining the RS assay. At a subsequent visit, the assay result and final treatment recommendations were discussed and physicians completed a second questionnaire on the final treatment plan. The statistical assumption of the study was based upon anticipating an overall treatment decision change rate (from chemo-hormonal therapy to hormone only, or vice-versa) to be at least 30%.

Results:
Out of 84 pts enrolled, 82 went on to have RS testing. 57% of pts had T1 tumors, 40% T2 and 2% T3 tumors. The majority of pts (77%) had N1 macroscopic disease compared to 23% that had microscopic nodal involvement. 63% of cases were grade 2, followed by 23% grade 1 and 14% grade 3. 60% of RS were low (<18), 33% intermediate (18-30) and 7% were high (>30). In 53% of cases there was an altered treatment plan (49% switched from chemo-hormonal therapy to hormone only, and 4% switched from hormone only to chemo-hormonal therapy). Of the 49% that switched to hormone only, 68% had a low RS and 33% had an intermediate RS. All 4% that switched to chemo-hormonal therapy had an intermittent RS.

Conclusions:
This multi-centered Canadian study showed that in 53% of cases, treatment recommendations were changed based on the RS. This suggests that there is clinical utility for the RS in 1-3 N+, ER+, and HER2- breast cancer.
Title: A predictive test for neoadjuvant chemotherapy in breast cancer identifies a subset of triple negative patients with resistant disease and the poorest prognosis

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Body: Prediction of pathological complete response (pCR) for neoadjuvant treatment is an area of unmet clinical need, especially for triple negative breast cancer (TNBC) as pCR is correlated with better outcomes. Predicting which patients will have residual disease (RD) provides an opportunity to improve treatment planning. We developed a test to predict which patients are likely to achieve pCR or RD to the standard of care (taxane-based) neoadjuvant chemotherapy using gene expression profiling of 325 previously identified novel biomarkers.

Three microarray datasets were used (GSE22226, GSE25055, and GSE25065) including a total of 594 stage II-III breast cancer patients of which 125 (21%) achieved pCR, and 469 (79%) RD. ER+ tumors were present in 57% of the patients and 52% were PGR+. Almost 90% of the patients were Her2-. Of 231 TNBC, 78 (33.8%) achieved pCR, while 153 (66.2%) RD. Of 303 ER+Her2-patients 26 (8.6%) achieved pCR while 277 (91.4%) RD. The cohort was divided into balanced populations with 476 patients used for training (80%) and test (20%) rounds of model development, while 118 patients were reserved as a validation set. Combining a “winnowing” process to remove genes with least predictive power, and hundreds of thousands of step-wise runs, followed by ranking genes based on conditional probabilities, we developed a 17-gene cassette (BA100) which was locked-down in the validation set with ROC (AUC) = 0.818. With a cut-off of 83% sensitivity and 68% specificity (PPV 0.4; NPV 0.94), BA100 achieved a 16% true positive rate (true pCR) and 55% true negative rate (true RD) identifying 76% of the patients who achieved pCR, and 69% of the patients with RD. In TNBC, BA100 classified 29% as true positives (TP), 36% as false positive (FP), 30% true negative (TN), and 4.8% false negative (FN). Kaplan Meier (KM) curves showed a significant difference in 5-year disease-free survival (5Y DFS) between TP and TN (p=0.00453) or FP (p=2.09E-06). However, FP had even worse outcomes than TN patients. To improve the TP rate, additional genes expressed in TNBC plus the original 325 genes were subjected to a second round of gene selection to discriminate between TP and FP, resulting in a 16-gene cassette (BA100.1). With a cut-off of 95% sensitivity and 73% specificity (PPV 0.7; NPV 0.95), applying BA100.1 reduced the FP rates from 24% to 9%, while correctly identifying 88% of RD in the validation set. KM curves showed no significant difference in 5Y DFS between 124 TNBC (53.7%) classified as TN versus 29 TNBC (12.6%) classified as FP, while a significant difference in survival rate was found between TNBC classified as TN vs TP (Cox Proportional Harzard p=8.42e-05).

Taken together, we developed a predictive test consisting of two gene cassettes that accurately identified 71% (88/104) of pCR, and 88% (417/469) of RD patients. Gene cassettes include several transcriptional repressors, PI3K signal transduction, components of telomerase, DNA repair genes, fatty acid metabolism and estrogen-independent proliferation. The test stratified TNBC with differential response to chemotherapy and survival rates so that novel approaches can be used without delay. Further validation will confirm the test utility.
Clinical correlations of serum vitamin D in patients undergoing neoadjuvant systemic therapy and survival outcomes for operable breast cancer

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Body: Introduction: There has been increasing interest in the potential benefit of vitamin D to improve breast cancer outcomes. Pre-clinical studies suggest vitamin D enhances chemotherapy-induced cell death. We previously reported (Thomas A SABCS 2016) that low serum vitamin D levels were associated with not attaining a pathologic complete response (pCR) following breast cancer neoadjuvant chemotherapy (NAC). We report here the impact of vitamin D on survival parameters in an expanded cohort of patients.

Methods: Patients from two Iowa registries who had serum vitamin D level measured before or during NAC were included. French patients enrolled in a previous study of the impact of NAC on vitamin D and bone metabolism were also eligible for this study. Vitamin D deficiency was defined as < 20 ng/mL. pCR was defined as no residual invasive disease in breast and lymph nodes. Survival was defined from the date of diagnosis to the date of relapse (PFS) or date of death (OS).

Results: The study included 327 women. Median age was 51 years. Patients presented with HER2+, HR+/HER2- and triple negative (TN) tumors in 28.5%, 43.9% and 27.6% of the cases respectively. In this expanded cohort, vitamin D deficiency remained associated with the odds of not attaining pCR (OR 1.64; 95%CI: 1.02-2.66, p=0.04). Median follow-up was 5.33 years (range 0.5 to 9.8 years). Of these patients, there were 54 relapse and 52 deaths. In multivariate analysis, stage III disease, TN phenotype and the inability to achieve pCR were independently associated with a worse survival (Table 1).

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<td>Non-pCR</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>pCR</td>
<td>0.19 (0.08 – 0.45)</td>
<td>&lt; 0.001</td>
<td>0.22 (0.09 – 0.55)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Vitamin D</strong></td>
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<td></td>
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<tr>
<td>&lt; 20 ng/mL</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>≥ 20 ng/mL</td>
<td>1.01 (0.57 – 1.78)</td>
<td>0.97</td>
<td>1.03 (0.58 – 1.84)</td>
<td>0.92</td>
</tr>
</tbody>
</table>

Vitamin D deficiency was not significantly associated with survival parameters in the general population; however a trend was seen in the TN population regarding the correlation with PFS (90 patients, 5-year PFS 60.4% vs. 72.3%, p=0.18).
Conclusion: Vitamin D deficiency is associated with the inability to reach pCR in breast cancer undergoing NAC. A trend for worse survival was seen in the TN subgroup. The strong association between vitamin D deficiency and the inability to reach pCR warrant further evaluation in the TN subgroup. Prospective interventional studies are needed to elucidate if maintaining vitamin D levels during NAC, a highly modifiable variable, may be utilized to improve cancer outcomes, particularly in TN breast cancers.
Title: Oncotype Dx testing in patients with synchronous unilateral primary breast cancer

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Body: Introduction: Oncotype DX, is a commercial diagnostic test that is used to predict the likely benefit from chemotherapy in ER+, HER2-negative, node-negative breast cancer. Patients with multiple, synchronous ipsilateral primary breast cancers often have >1 tumor tested if results from the first tumor show low or intermediate recurrence scores. Whether this approach is cost effective is unknown.

Methods: We reviewed Oncotype Dx results for 907 ER+/HER2- breast cancer patients seen at Rush University Medical Center from 2/2006 to 10/2016. Thirty-nine patients (35 with the same histology) had multiple, synchronous unilateral tumor samples tested. Results were reported both on the numeric risk score associated with the test and the categorized result (low/intermediate/high) recurrence risk. For patients undergoing more than 2 tests, the first two were arbitrarily chosen for the paired analysis. Descriptive statistics were used to examine the risk score distributions and assess potential correlation between paired samples. Statistical inference methods included estimating the correlation coefficient, regression models predicting one score with another, and evaluating the paired score differences with respect to mean deviation from zero. For categorized risk score, analysis evaluating category concordance between tumors was used to assess the degree of agreement. Analyses were conducted on all patients, and a subset consisting of patients whose two tumors had the same histology, where scores may be expected to exhibit greater concordance.

Results: Categorical risk score (RS): The concordance rate for risk category was 77% (30/39 patients) and Kendall’s tau measure of association = 0.57, and was further increased to 80% (28/35 patients) and tau 0.63 for tumors with the same histology. Only one patient had tumor samples with low and high RS. A second patient had tumor samples with intermediate and high RS. Continuous RS: The correlation was 0.81 (p < 0.0001). For tumors with the same histology, correlation increased to 0.83, with regression $r^2$ (predicting second tumor RS value with first) = 0.69, indicating that the majority of variation in second score can be explained by variation in first score.

Conclusion: Oncotype Dx testing in patients with synchronous, unilateral primary breast cancers generally results in concordant results, even more so when the tumor samples are of the same histology. In our study, only 1 of the 39 patients had discordant low/high tumor pairs. Continuous scale tumor RS scores were highly correlated (> 0.8). Therefore the benefit of testing additional samples appears marginal and, with the $4,620 cost per test, may not be cost-effective.
Title: A microRNA signature associated with pathological complete response to a novel neoadjuvant therapy regimen in triple negative breast cancer

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Body: Background: Locally advanced triple negative breast cancer (laTNBC) patient’s exhibits resistance to neoadjuvant chemotherapy (NC) and poor survival. Distinct therapeutic combinations have been used to reduce high mortality. Recently, novel regimens of NC for laTNBC have achieved pathological complete response (pCR) rates of 10-50%. Evaluation of pCR during oncologic treatment is decisive to identify those patients that response or not response to NC. MicroRNAs (miRNAs) are small non-coding RNAs that represent novel and potential predictive biomarkers useful to identify the patients who will get pCR in cancer.

Methods: Thirty-five patients diagnosed with laTNBC, were invited to participate in this study and enrolled after they signed an informed consent. The 22 patients with pCR and 13 patients without pCR received the experimental NC fluorouracil, adriamycin, cyclophosphamide, cisplatin, paclitaxel (FAC--CDDP/paclitaxel). MiRNA expression profiling was evaluated for 754 miRNAs. A discovery cohort (n=10 pCR and n=8 no-pCR) and a validation cohort (n=12 pCR and n=5 no-pCR). Bioinformatics analysis revealed the affected cellular pathways in pCR group. After a median clinical follow-up of 60 months, statistical analysis was performed to identify miRNAs that could discriminate pCR from no-pCR by using FAC--CDDP/paclitaxel.

Results: MiRNAs expression profiling identified 11 miRNAs that showed significant differences between pCR and no-pCR (p<0.05 and fold change >1.5) groups. Eight miRNAs (miR-9-3p, -30a-3p, -135b, -135b*, -380-5p, -941, -652 and miR-181c*) were upregulated and three miRNAs (miR-770-5p, -584 and miR-143) were downregulated in pCR patients. The altered cellular pathways for the set of miRNAs were PI3K/AKT, FoxO, Ras and ERBB (p<0.05). Four differentially expressed miRNAs (miR-770-5p, miR-143-5p, miR-30a-3p, miR-9-3p) were confirmed in the validation phase. Expression of these miRNAs above the median level was a significant predictor of pCR to experimental NC in laTNBC patients (p<0.001).

Conclusions: These four validated miRNAs could be used as predictors of pCR in response to FAC--CDDP/Paclitaxel treatment in laTNBC patients.
Title: Effects of radiotherapy on breast cancer outcomes among stage I, low-Recurrence risk, hormone-Sensitive breast cancer: Pooled analysis of individual data from phase III trials

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Body: Purpose: Radiotherapy after breast conservation has become the standard of care. Prior meta-analyses of radiotherapy benefits pre-dated availability of gene expression profiling (GEP) to assess recurrence risk and/or did not include all relevant outcomes. This analysis utilized GEP information with individual-level data from seven clinical trials to evaluate the impact of omitting radiotherapy on recurrence and mortality.

Patients and Methods: The pooled observational data included women who had undergone breast conservation surgery for stage I, ER+ and/or PR+/HER2- cancers with clinicopathologically estimated 21-Gene Recurrence Scores (RS) of ≤18, and did not receive chemotherapy (n= 1,684). The primary endpoint was 10-year invasive or DCIS recurrence free-interval (IRFI-DCIS), including breast cancer death and was estimated using adjusted Cox models. Secondary outcomes were breast-cancer specific and all-cause survival. Covariates included for all models were age, tumor size, grade, hormonal status, type of hormonal therapy, risk score, and trial.

Results: Ten-year IRFI-DCIS was high (96.6% with radiotherapy vs. 91.6% without), for an absolute difference of 5%. Omission of radiotherapy (vs. radiotherapy) was associated with an overall adjusted hazard ratio of 2.6 (95% confidence interval 1.5-4.5) for any first event. There was only a significant increase in risk of loco-regional; not, distant recurrence, breast cancer-specific or overall survival. The effect of radiotherapy varied across subgroups, with lower first event rates for those with estimated RS of <11 (vs. 11-18), and women ages 60+ (vs. <60 years).

Conclusion: Omission of radiotherapy in hormone-sensitive patients with low recurrence risk may lead to a modest absolute increase in loco-regional recurrence, but does not appear to increase risk of distant recurrence or death.
Title: Three-year outcomes with hypofractionated (HF) versus conventionally fractionated (CF) whole breast irradiation (WBI)

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Body: Background: Adoption of HF-WBI remains low due in part to concerns regarding its safety when used with a tumor bed boost or in patients who have received chemotherapy or have large breast size. To address this, we conducted a randomized, multicenter trial to compare CF-WBI (50Gy/25fx+10-14Gy/5-7fx) to HF-WBI (42.56Gy/16fx+10-12.5Gy/4-5fx).

Methods: From 2011 and 2014, 287 women with stage 0-II breast cancer, age ≥40 years, were randomized to CF-WBI or HF-WBI, stratified by chemotherapy and breast size. The primary outcome was the proportion of patients with adverse Breast Cancer Treatment Outcomes Scale (BCTOS) cosmetic score three years post-WBI, defined as a score of ≥2.5 (a score of 1 indicates no difference between the treated breast and contralateral breast, 2-slight difference, 3-moderate difference, 4-large difference). Secondary patient-reported outcomes included BCTOS functional status, BCTOS breast pain, Body Image Scale (BIS), and the FACT-B Trial Outcome Index (TOI). Additional secondary outcomes included photographically-assessed cosmesis scored by a 3-physician panel blinded to randomization arm using the RTOG scale, physician-assessed CTCAEv4.0, overall survival (OS), local recurrence-free survival (LRFS) and distant metastasis-free survival (DMFS). Chi-square, Fisher's exact, Wilcoxon's rank sum, logistic regression, and log-rank tests evaluated differences by treatment arm; survival was measured using the Kaplan-Meier method. All analyses were intention-to-treat.

Results: Of 287 patients enrolled, 286 received the protocol-specified radiation dose. Thirty percent received chemotherapy (73% anthracycline, 92% taxane, 22% trastuzumab), 37% had large breast size (D cup or larger), and 44% were obese (BMI≥30). All baseline characteristics were well-balanced by treatment arm (P>0.05). Median follow-up is 4.1 years. For the primary outcome, adverse BCTOS cosmetic score at 3 years was noted in 13.6% (n=15/110) of CF-WBI and 8.2% (n=8/97) of HF-WBI patients (P=0.22). There were trends for lower risk of adverse BCTOS cosmetic score with HF-WBI, compared to CF-WBI, among patients who did not receive chemotherapy (OR=0.25, 95%CI 0.06-1.03; Pinteraction=0.07) or with large breast size (OR=0.26, 95%CI 0.07-0.98; Pinteraction=0.08). There were no patient subgroups where risk of adverse BCTOS cosmetic outcome was significantly higher with HF-WBI compared to CF-WBI. For secondary outcomes, there was no difference by treatment arm for BCTOS functional status (P=0.83), BCTOS breast pain (P=0.69), BIS (P=0.45), or FACT-B TOI (P=0.79). Poor-fair cosmetic outcome at 3 years assessed by the 3-physician panel was noted in 28.8% of CF-WBI patients and 35.4% of HF-WBI patients (P=0.31). In total, 19% CF-WBI patients and 20% of HF-WBI patients had a grade 2-3 toxicity at the three-year evaluation, with no difference by treatment arm (P=0.84). Five-year OS, LRFS, and DMFS were 99%, 98%, and 99%, respectively, with no difference by treatment arm (P=0.68, 0.37, 0.62).

Conclusions: Three years after WBI followed by a tumor bed boost, outcomes with HF and CF are similar. Tumor bed boost, modern chemotherapy, and large breast size do not appear to be clinically meaningful contraindications to HF-WBI.
**Title:** Phase 0-I prospective study of proton beam radiation for locally advanced breast cancer

Rachel B Jimenez, Beow Yeap, Shea Hickey, Nicolas DePauw, Estelle Batin, Alphonse G Taghian, Hsiao-Ming Lu and Shannon M MacDonald. 1Massachusetts General Hospital, Boston, MA.

**Body:**

**Purpose/Objectives:**
The aim of this phase 0/I study was to assess the feasibility of proton beam therapy for locally advanced breast cancer (LABC) following mastectomy.

**Materials/Methods:**
Patients > age 18 with breast cancer managed with mastectomy +/- reconstruction and requiring postmastectomy radiation (RT) were eligible if: 1) breast reconstruction prevented adequate target coverage or 2) ≥ 5% of the heart received ≥ 20 Gy and/or LAD received ≥ 20 Gy by conventional planning. RT consisted of passively scattered (3D-CPT) or pencil beam scanning (PBS) RT to the chest wall and internal mammary nodes (IMN) +/- other regional lymphatics. The primary endpoint was the incidence of grade 3 radiation pneumonitis (RP) or any grade 4 toxicity within 3 months following completion of treatment. Secondary endpoints included 5 year rate of local regional control (LRC) and overall survival (OS), acute skin toxicity per CTCAE 4.0, and acute and late toxicity of breast reconstruction, including any unplanned surgical intervention due to contracture, infection, or cosmesis. In addition, patients underwent cardiac strain echocardiograms and blood-based cardiac biomarkers (MPO, pro-BNP, ultrasensitive troponin-I) prior to RT and at 4 and 8 weeks after RT to assess subclinical cardiac changes.

**Results:**
64 patients were enrolled and completed RT between 2011-2016. 23 patients received 3D-CPT through 2012 after which 41 subsequent patients received PBS. Median age was 45 years (IQR: 39-50 years). Among participants, 59 (92%) were left-sided, 2 were bilateral, and 3 were right-sided. 98.4% (n=63) received systemic chemotherapy/HER2-based therapy and of these, 56.3% (n=36) received neoadjuvant therapy. Among neoadjuvant patients, 2.8% (n=1) were clinical stage I, 67.7% (n=24) were stage II, and 30.6% (n=11) were stage III. Of 28 patients who received upfront surgery, 17.9% (n=5) were pathologic stage I, 42.9% (n=12) were stage II, and 39.3% (n=11) were stage III. 48 patients (75%) underwent immediate reconstruction. Median dose to the chest wall was 50.4GyE (range: 46.8GyE-52GyE) Median dose to the IMN was 50.4GyE (range: 45GyE-52GyE). At a median follow-up of 2.9 years among 60 living patients, LRC 3: 98% / OS 3: 92%. 1 3D-CPT patient developed grade 2 RP 4 months after RT, none developed grade 3 RP. There were no grade 4 toxicities. Acute skin toxicity was similar between modalities with 3D-CPT grade 1/2/3=4%/91%/4% versus PBS grade 1/2=15%/85% (p=0.41). Unplanned surgical re-intervention at 3 years was 17% for 3D-CPT and 28% for PBS (p=0.28).

**Conclusions:**
Proton beam RT (3D-CPT or PBS) is safe and feasible for post-mastectomy patients. Imaging and blood-based analysis of early cardiac changes in this cohort is in process, but preliminary assessment of the first 30 patients shows no decrement in cardiac strain. Randomized clinical data is needed to determine whether there are long-term clinical benefits of proton beam RT in LABC patients.
Comparing whole heart versus coronary artery dosimetry in predicting the risk of cardiac toxicity following breast radiation therapy

Sagar A Patel¹, Syed S Mahmood², Trinh Nguyen¹, Beow Y Yeap¹, Rachel B Jimenez¹, Alphonse G Taghian¹, Nandini M Meyersohn³, Tomas G Neelan¹ and Shannon M MacDonald².¹ Massachusetts General Hospital, Boston, MA; ²Massachusetts General Hospital, Boston, MA and ³Massachusetts General Hospital, Boston, MA.

Body: Purpose: Prior localization studies have implicated accelerated left anterior descending artery (LAD) atherosclerosis in the development of radiation-induced heart disease following breast radiation therapy (RT). However, whole heart dose constraints, namely mean heart dose (MHD), are most commonly utilized in RT treatment planning to predict and avoid cardiac toxicity. This study compares the relationship between MHD and LAD maximum dose (Dmax) to the onset of coronary artery disease identified on CT angiogram (CTA).

Methods: We identified 52 women with stage I-III breast cancer (36 left, 16 right) treated with adjuvant breast or chest wall 3-dimension conformal RT who subsequently underwent CTA. No cardiac substructural dose constraints were used at treatment, and coronary vessels were contoured retrospectively and reviewed by a cardiac radiologist. Dosimetry to the LAD was calculated based on the individual RT plan used for treatment. A nested case-control study of incident LAD stenosis ≥ 25% luminal involvement and coronary artery calcification (CAC) using Agatston score was conducted. Controls were matched to cases on elapsed years between RT and CTA. Odds ratios (OR) were calculated using conditional logistic regression to assess the attribution of LAD Dmax and MHD dose parameters, adjusting for Atherosclerotic Cardiovascular Disease (ASCVD) score, history of ischemic heart disease, and statin use.

Results: The median follow-up time from RT to CTA was 5.1 years (range, 2.5-18.1). LAD Dmax was more strongly associated with the onset of any CAC (Agatston score ≥1), moderate/severe CAC (Agatston score ≥101), and LAD stenosis (≥25% lumen). For any CAC, OR was 1.15 (95% CI 0.99-1.33, p=0.06) and 2.21 (95% CI 1.13-5.03, p=0.02) for MHD and LAD Dmax, respectively. For moderate/severe CAC, OR was 1.04 (95% CI 0.95-1.23, p=0.24) and 2.57 (95% CI 1.01-7.04, p=0.04) for MHD and LAD Dmax, respectively. For LAD stenosis, OR was 1.21 (95% CI 1.01-1.46, p=0.04) and 4.85 (95% CI 1.42-16.63, p=0.01) for MHD and LAD Dmax, respectively. LAD Dmax > 10 Gy was a significant threshold for increased odds of developing any CAC (OR 10.21, 95% CI 1.42-21.83, p=0.03), moderate/severe CAC (OR 5.21, 95% CI 1.16-18.36, p=0.04), and LAD stenosis (OR 6.52, 95% CI 1.39-19.67, p=0.03).

Conclusions: Compared to MHD, LAD Dmax had a stronger association with the onset of CAC and LAD stenosis identified on CTA. The LAD should be more routinely included as an avoidance structure for breast RT planning, and if confirmed on prospective analysis, a Dmax threshold of 10 Gy may serve as a useful clinical parameter to minimize late cardiac toxicity.
Title: Long-Term outcomes using electron IORT APBI for early stage breast cancer: The Verona University Hospital experience

Nunzia Luna Valentina Cernusco, Nadia Marcia, Mario de Liguoro, Maria Grazia Giri, Stefania Guariglia, Davide Lombardi, Francesca Pellini, Carlo Cavedon, Giovanni Paolo Pollini and Renzo Mazzarotto. 1Radiation Therapy Unit-Azienda Ospedaliera Universitaria Integrata di Verona, Verona, Veneto, Italy; 2Medical Physics Unit-Azienda Ospedaliera Universitaria Integrata di Verona, Verona, Veneto, Italy and 3Breast Unit-Azienda Ospedaliera Universitaria Integrata di Verona, Verona, Veneto, Italy.

Body: Purpose: Several published studies of electron intraoperative radiation therapy (IORT), when delivered as accelerated partial breast irradiation (APBI), have demonstrated that low recurrence rates of 1.5-2.5% are achieved at 5-year follow-up for ASTRO Suitable or ESTRO Good women. There has been no report on IORT APBI with longer follow-up to determine if these excellent low recurrence results persist. We evaluated our patient cohorts treated with IORT APBI, with follow-up up to 10 years, to answer the question of long-term control.

Methods and materials: From July 2006 to December 2015, 295 patients suitable for breast-conserving therapy (BCT) entered a single-arm phase II study and were treated with IOERT, as radical treatment, immediately after surgical resection using a single dose of 21 Gy to Dmax. Patient inclusion criteria were mostly ASTRO Suitable Group except for G3 grade. All patients received IOERT with a dedicated mobile linear accelerator (Mobetron, Sunnyvale, CA). A Lucite disk was placed under the gland to protect the chest wall. For each patient, the field size was selected primarily on the tumor dimension, and the applicator was chosen with a diameter that provided a 2-cm lateral margin.

Results: With a median follow-up of 60 months (1-122 months), 4 women (1.3%) experienced a true local recurrence (reappearance of the tumor in the same quadrant) diagnosed 1 year after the first treatment in 1, 3 years after in 2, and 8 years after in 1 patient. None of the patients had a new ipsilateral carcinoma (reappearance of cancer in another quadrant of the same breast). In addition, 2 women (0.67 %) developed distant metastases, 2 (0.67%) subsequently developed contralateral breast cancer, and 9 (3%) other cancers. 21 patients (7.1%) died, but only 2 (0.67%) due to breast cancer systemic progression. Patients that recurred underwent mastectomy. One woman, who developed contralateral breast cancer, was treated with IORT APBI.

Conclusion: Single-dose IOERT in early stage breast cancer can be delivered safely and with excellent results. Our long-term recurrence rates are very low and thus encourage the use of IORT in selected low risk patients even off-clinical trials.
Title: Analysis of cardiac events among node positive breast cancer (NPBC) patients treated with three-dimensional conformal radiation therapy (3D-CRT)

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Body: Background: Regional nodal irradiation (RNI) in addition to the chest wall and/or breast can maximize local regional control and improve overall survival, but has been associated with late cardiac morbidity. We examined NPBC patients treated with RNI using 3D-CT based radiation therapy (RT) to evaluate incidence and type of cardiac events.

Methods: Between 2000 and 2007, 156 NPBC patients were treated with RNI following lumpectomy or mastectomy using 3D-CRT. In all cases, treatment target and normal tissue volumes were delineated on treatment CT scans. The heart contour included the left ventricle and the atria. Prescription dose was typically 50Gy in 25 fractions (range 44-54 Gy) to the chest wall and/or breast PTVeval. 37% received a boost to the chest wall and 73% to the lumpectomy cavity. The mean prescription dose to the axilla and supraclavicular lymph nodes was 47.6 Gy (range 43.2 – 54 Gy) and 46.8 Gy to the IMN (range 35.3 – 50.4 Gy). The dose-volume cardiac data and incidence of cardiac events is reported.

Results: Median follow-up of surviving patients was 7 years (range, 0.3-10.6). Median patient age was 50 (range, 27-91), 52% were premenopausal, 76% estrogen receptor positive, and 18% were HER-2 positive. The IMN received > 40 Gy in 66%. Chemotherapy was used in 94% of patients, and it was anthracycline-based in 82.3%. At the time of RT, 12.5% smoked, 9% had diabetes, 33% with HTN, and 4.4% had a history of CAD.
Average mean heart dose for the cohort was 5.2 Gy (range, 0.2 - 25.3 Gy). Mean cardiac V25 was 5.4% (range, 0-20%), mean cardiac V45 was 1.7% (range, 0-13.3%), and mean maximum cardiac point dose was 45.4 Gy.
There was 1 (0.7% of cohort) right sided patient with cardiac events and 8 (5.1% of cohort) left experiencing cardiac events. A total of 18 cardiac diagnoses were experienced among the 9 patients: Coronary artery disease with or without myocardial infarction (4), congestive heart failure (6), cardiomyopathy (3), and arrhythmia (5).

Conclusions: The cardiac event rate among these NPBC patients treated with RNI and anthracycline-based chemotherapy was low, but more common in women with left-sided breast cancer compared to right. Additional analysis using 3DCRT volumes are important to validate these findings and better define the dose-volume parameters for cardiac toxicity.
Title: Evaluation of lung and heart dose in patients treated with radiation for breast cancer

Erin H Healy¹, David N Pratt¹, Dominic DiCostanzo¹, Jose G Bazan¹ and Julia White¹. ¹The Ohio State University Medical Center, Columbus, OH.

BACKGROUND: A recent systematic review of women receiving radiation therapy (RT) for breast cancer combined with modeled estimated risks of mortality from heart disease and lung cancer found that the mean heart dose (MHD) was 4.4 Gy (5.2 Gy for left-sided, 3.7Gy for right-sided) and the mean total lung dose (TLD) was 5.7 Gy. Estimated excess cardiac mortality ranged from 0.3-1.2% and lung cancer mortality ranged from 0.2-4.4% with modern RT. Using these data as a benchmark, we set to review the MHD and mean TLD for our patients receiving adjuvant breast RT in a modern era when RT planning includes meeting normal tissue constraints.

METHODS: We evaluated the MHD and mean TLD for patients with unilateral breast cancer treated with curative intent between January 2012 and May 2017 at our institution. Dosimetric data was complete for 793 patients. During this time period the MHD constraint was 4 Gy and lung V20 was 20% for breast only and 35% for regional nodal irradiation (RNI). RNI included the axillary, supraclavicular and internal mammary nodes. Patients were evaluated by laterality (right vs. left), prone vs. supine position, breast only whole breast irradiation (WBI) and RNI with intact breast or chestwall post-mastectomy. The RNI group was examined by treatment technique, intensity modulated radiation therapy (IMRT) vs. 3D conformal (3DCRT). We compared differences in the MHD and mean TLD within those groups using the Student's t-test.

RESULTS: We identified 651 patients: 481 WBI only and 170 RNI. In the RNI group, 77 (45.3%) received IMRT. Of the WBI only group, 229 (47.6%) were right-sided and 313 (65.1%) were treated prone. The mean TLD for the WBI only group was significantly lower in the prone vs. supine position (0.62 Gy vs. 3.90 Gy, p<0.0001). The prone position resulted in lower MHD for both left-sided WBI (1.17 Gy vs. 1.67 Gy, p<0.0001) and right-sided WBI (0.51 Gy vs. 0.64 Gy, p=0.1067). In patients that received RNI, the mean TLD was 8.20 Gy (SD 1.03) and the MHD was 2.67 Gy (3.25 Gy for left-sided vs. 1.83 Gy for right-sided, p=0.0001). Compared to 3DCRT, IMRT increased the MHD (2.46 Gy vs. 4.23 Gy for left-sided, p<0.0001; 0.94 Gy vs. 2.85 Gy, p<0.0001 for right-sided) and mean TLD (8.50 Gy vs. 7.95 Gy, p=0.0005).

CONCLUSIONS: In the era of RT treatment planning that incorporates normal tissue constraints, very low MHD and lower TLD are achievable in prone or supine position patients receiving WBI only for breast conserving treatment. This means lower late cardiac and lung cancer mortality risks from RT. Women that receive RNI also have acceptably low MHD but high mean TLD. Node positive breast cancer patients derive a disease free survival benefit from RNI, which must be balanced against potential late risk for lung cancer, especially in smokers. More attention should be focused on identifying lung cancer risk, smoking cessation and screening efforts in node positive breast cancer patients with indications for RNI to minimize late radiation risks.
Title: Risk factors for fibrosis after whole breast radiation therapy in lateral position : A large scale single center experience

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Body: Background and Purpose: We previously confirmed the efficacy and safety of our technique of whole breast radiation therapy (WBRT) in isocentric lateral decubitus (ILD) position. The purpose of this work is to evaluate the risk factors for long-term fibrosis in patients treated in ILD position.

Material and methods: We studies 832 consecutive female patients with early stage BC treated by conservative surgery followed by 3D-conformal WBRT at Institut Curie between 2005 and 2010. Fibrosis and deformation were evaluated at the end of the treatment and subsequently every 6 months during at least 5 years, using NCI CTC v3.0 scale. Different fractionation schedules were used: 66Gy in 33 fractions, 50Gy in 25 fractions, 40Gy in 15 fractions, 41.6Gy in 13 fractions and 30Gy in 5 weekly fractions.

Results: Median age is 61.5 years (range: 29 - 90); median follow up is 6.4 years (range: 1.5 - 12.4). During the follow-up, 308 patients (38.9%) had grade 1 fibrosis, and 36 patients (4.3%) only had grade 2-3 fibrosis. Among patients with grade 1 to 3 fibrosis, the median time to development of fibrosis was 1.6 years (range: 9 days – 8.3 years). In univariate and multivariate analysis, age, cup size and chemotherapy administration had no significant influence on development of breast fibrosis. The hypo fractionated schedule of 30Gy in 5 fractions significantly increased the fibrosis rate (OR=12.5; [2.7; 57.1] p=0.001). On the other hand, 40 Gy/15 fr and 41.6 Gy/13 fr had no significative influence (OR=2.2 [0.5; 11.1] p=0.32) as well as the 66 Gy/33 fr schedule (OR=3.6 [0.8; 15.4] p=0.09) compared to standard scheme of 50 Gy/25 fr. The cosmetic result was good or excellent for 84.8% of cases at the first evaluation. The 30 Gy in 5 fractions schedule significantly influenced the cosmetic result in the multivariate analysis (OR=11.2 [3.1; 43.9]; p<0.001), with increased rates of breast deformation. The risk of breast deformity was also worse for large breast size (OR=2.7 [1.3; 5.8]; p=0.02).

Conclusion: Whole breast radiation therapy in ILD position is well tolerated with good cosmesis and low rates of fibrosis, except for the 30 Gy in 5 weekly fractions schedule. Large breast size has a significantly negative influence on cosmetic results and fibrosis.
**Title:** Excellent acute toxicity outcomes with proton therapy for partial breast irradiation in early stage breast cancer: Initial results of a multi-institutional phase II trial

J Isabelle Choi¹ and Andrew L Chang¹,². ¹Scripps Proton Therapy Center, San Diego, CA and ²ProCure Oklahoma City, Oklahoma City, OK.

**Body:**

**Background and Purpose:** Partial breast irradiation (PBI) with proton therapy after lumpectomy for early stage invasive breast cancer is an area of active investigation. Advantages of this technique include a shorter treatment course and the potential for decreased morbidity versus external beam photon radiation therapy given superior sparing of the surrounding normal breast tissue. To date, multiple single-institutional studies have reported conflicting results on the acute toxicity of PBI. This prospective phase II trial investigates the feasibility, safety, and efficacy of delivering PBI with proton therapy in a multi-institutional setting.

**Methods:** Patients over the age of 50 years with ER-positive nonlobular invasive breast cancer or ductal carcinoma in situ ≤3 cm in size who had undergone lumpectomy with at least 2 mm negative surgical margins were treated with proton therapy to a dose of 40 Gy delivered over 10 daily fractions. In this initial analysis, we assess early toxicity and treatment efficacy of proton PBI. Patients were followed up at 4 weeks post-treatment and annually thereafter, along with annual mammograms. Patient-reported quality of life and physician-reported cosmesis assessments including photographs were obtained at 1 and 3 years post-treatment.

**Results:** Forty patients were enrolled, of which 38 were evaluable. At a median follow-up of 17.8 months (range 2-36 months), all patients had overall breast cosmesis that was scored “good” or “excellent”. Of 6 grade 2 acute adverse events that occurred, only 1 was radiation dermatitis, with others including lymphedema, hot flashes, and fatigue. One grade 3 acute toxicity occurred 3 weeks after radiation completion in the form of vascular disease requiring stent placement, highly unlikely to be attributable to radiation effects. Patient-reported quality of life outcomes were recorded using the standardized Breast Cancer Treatment Outcome Scale (BCTOS) scored from 1-4 (1: none; 2: mild; 3: moderate; 4: large), with endpoints receiving a score of 3 or 4 most frequently involving change in breast size, breast texture, nipple appearance, or scar tissue. Patients assigned a score of 4 for change in nipple appearance (n=2), breast shape (n=2), and scar tissue formation (n=2). To date, local, locoregional, and distant disease control are 100%, although one patient has developed a new hormone receptor-negative invasive ductal carcinoma of the contralateral breast.

**Conclusion:** Proton PBI provides excellent early cancer control with acceptable cosmetic outcomes and minimal adverse effects as per patient- and physician-reported assessments. On continued follow-up, late toxicity and cosmesis, as well as long-term disease control outcomes, will be assessed.
Title: Toxicity analysis of elderly breast cancer patients using different accelerated partial breast irradiation techniques

Daphne HM Jacobs, Corrie AM Marijnen, Gabrielle Speijer, Marieke Straver, Andreas Marinelli, Jos Merkus, Ellen MA Roelofzzen, Lida AG Zwanenburg, Ursula Fisscher, Anna L Petoukhova, Mirjam E Mast and Peter Koper.

1 Leiden University Medical Center, Leiden, Netherlands; 2 Haga Hospital, The Hague, Netherlands; 3 Haaglanden Medical Center, Leidschendam, Netherlands; 4 Haga Hospital, The Hague, Netherlands; 5 Isala Clinics, Zwolle, Netherlands and 6 Haaglanden Medical Center, Leidschendam, Netherlands.

PURPOSE: We investigated the acute toxicity of Accelerated Partial Breast Irradiation using external beam (EB-APBI) and intraoperative radiotherapy (IORT) technique.

MATERIALS AND METHODS: Women ≥60 years with unifocal breast tumors of ≤30 mm undergoing breast conserving therapy were eligible for this prospective multi-centre cohort study. After informed consent, patients were treated with IORT in one institute, EB-APBI was applied in other institutes. IORT was applied with electrons directly on the tumor bed following lumpectomy (21 Gy at 90% isodose). EB-APBI was delivered using 3D-conformal radiotherapy or IMRT in 10 daily fractions of 3.85 Gy. Acute toxicity was scored using the CTCAE v.3, initially by the treating physician and retrospectively by blinded researchers. The highest reported toxicity until 3 months after treatment was used. Patient reported symptoms were analysed using Visual Analogue Scales (VAS) for pain and fatigue (scale 0-10), and single items from the EORTC QLQ-C30 and Breast Cancer module questionnaires at different time points up till 3 months. P<0.01 was deemed significant.

RESULTS: Between January 2011 and August 2016, 622 patients were eligible, eventually 268 patients underwent IORT and 207 underwent EB-APBI. Of those, 267 (IORT) and 206 (EB-APBI) were available for toxicity analysis. Patient characteristics are shown in table 1. Although more patients experienced >grade 2 acute toxicity in the IORT group (10.4% IORT and 4.9% EB-APBI; p=0.027), grade 3 toxicity was low and did not significantly differ between groups (3.3% IORT and 1.5% EB-APBI). No grade 4 toxicity occurred. After IORT more wound infections requiring oral antibiotics (5.2% versus 1.5%) and more seroma requiring aspiration (2.2% versus 0.5%) were observed.

Results for EORTC symptoms and VAS scores are displayed in table 2. Patients treated with EB-APBI experienced significantly less fatigue direct postoperatively (EORTC p=0.003, VAS p=0.001). After 3 months, this difference dissolved, and only pain according to the VAS scale was significantly worse in the EB-APBI group (p=0.003).

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>IORT, n=267</th>
<th>EB-APBI, n=206</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median; range)</td>
<td>68; 59-90</td>
<td>67; 59-86</td>
<td>0.683</td>
</tr>
<tr>
<td>pT stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pTis</td>
<td>7%</td>
<td>13%</td>
<td>0.020</td>
</tr>
<tr>
<td>pT1</td>
<td>84%</td>
<td>74%</td>
<td></td>
</tr>
<tr>
<td>pT2</td>
<td>9%</td>
<td>13%</td>
<td></td>
</tr>
<tr>
<td>pN stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pN0</td>
<td>93%</td>
<td>97%</td>
<td>0.092</td>
</tr>
<tr>
<td>pN1</td>
<td>7%</td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td>ER status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>93%</td>
<td>93%</td>
<td>0.122</td>
</tr>
<tr>
<td>Systemic therapy</td>
<td>No</td>
<td>59%</td>
<td>0.509</td>
</tr>
</tbody>
</table>

Table 2. EORTC and VAS scores
<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Postoperative</th>
<th>After EB-APBI</th>
<th>3 months after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IORT</td>
<td>EB-APBI</td>
<td>EB-APBI</td>
<td>IORT</td>
</tr>
<tr>
<td>Pain</td>
<td>7</td>
<td>7</td>
<td>19</td>
<td>11</td>
</tr>
<tr>
<td>Fatigue</td>
<td>11</td>
<td>9</td>
<td>29</td>
<td>24</td>
</tr>
<tr>
<td>Breast pain</td>
<td>4</td>
<td>4</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td>Swollen breast</td>
<td>1</td>
<td>2</td>
<td>25</td>
<td>5</td>
</tr>
<tr>
<td>Oversensitive breast</td>
<td>3</td>
<td>3</td>
<td>36</td>
<td>15</td>
</tr>
<tr>
<td>Skin side effects</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>VAS pain(median)</td>
<td>3.0</td>
<td>3.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>VAS fatigue(median)</td>
<td>4.0</td>
<td>3.0</td>
<td>4.0</td>
<td>3.0</td>
</tr>
</tbody>
</table>

EORTC: Scores of “quite a bit” and “very much” were scored positive. Percentage of patients that scored positive is shown.

CONCLUSION Even though more clinically relevant toxicity is seen in IORT treatment direct postoperatively, acute toxicity in both IORT and EB-APBI treatment is acceptable. IORT patients seem to recover more rapidly regarding fatigue than EB-APBI patients. This may be explained by the fact that IORT treatment is completed in one day.
2017 San Antonio Breast Cancer Symposium

Publication Number: P2-11-11

Title: Role of postmastectomy radiotherapy after neoadjuvant chemotherapy in breast cancer patients: A study from the Japanese breast cancer registry

Minoru Miyashita¹, Naoki Niikura², Hiraku Kumamaru³, Hiroaki Miyata⁴, Takanori Ishida¹, Takayuki Kinoshita⁵, Hitoshi Tsuda⁶, Seigo Nakamura⁷ and Yutaka Tokuda². ¹Graduate School of Medicine, Tohoku University; ²Tokai University School of Medicine; ³Graduate School of Medicine, Tokyo University; ⁴Keio University; ⁵National Cancer Center Hospital; ⁶National Defense Medical College and ⁷Showa University.

Body: Background:
Postmastectomy radiotherapy (PMRT) has been shown to be beneficial in node-positive breast cancer patients. However, the role of PMRT for patients receiving modern neoadjuvant chemotherapy (NAC) are controversial. We aimed to evaluate the efficacy of radiotherapy for breast cancer patients treated with NAC and mastectomy in the Japanese Breast Cancer Registry.

Methods:
Patients who received NAC and mastectomy for cT1-4 cN0-2 M0 breast cancer were included in this analysis. We assessed locoregional recurrence (LRR), distant disease-free survival (DDFS), and overall survival (OS) using the Kaplan-Meier method and compared them between the groups with and without PMRT by nodal status after NAC; ypN0, ypN1, and ypN2-3. We also performed multivariable cox regression analysis to evaluate the association of radiotherapy and these outcomes adjusting for baseline patient and cancer characteristics.

Results:
Of the 145,530 patients registered from 2004 to 2009, we identified 3,226 patients who met our inclusion criteria with the 5-year follow-up information including 1,299 ypN0, 1,036 ypN1, and 879 ypN2-3 cases. PMRT was performed in 185 patients (14.2%) with ypN0, 265 patients (25.6%) with ypN1, and 543 patients (61.8%) with ypN2-3. There was no difference in LRR, DDFS, and OS between the groups with and without radiotherapy for ypN1 patients who received NAC (P=0.72, P=0.29, and P=0.36, respectively). For patients with ypN2-3 breast cancer, radiotherapy significantly improved LRR (P<0.001), DDFS (P=0.01), and OS (P<0.001) on univariate analysis. No difference in LRR, DDFS, and OS was observed for ypN0 patients (P=0.81, P=0.15, and P=0.05, respectively). In multivariable analysis, the use of radiotherapy was independently associated with improved LRR [hazard ratio (HR): 0.608, 95% confidence interval (CI): 0.452–0.818, P=0.001] and OS [HR: 0.685, 95% CI: 0.531–0.885, P=0.004] for ypN2-3 patients.

Effect of PMRT on locoregional recurrence by the ypN subgroups

<table>
<thead>
<tr>
<th>ypN subgroup</th>
<th>Hazard ratio</th>
<th>95%CI Low</th>
<th>95%CI High</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ypN0</td>
<td>0.855</td>
<td>0.458</td>
<td>1.596</td>
<td>0.623</td>
</tr>
<tr>
<td>ypN1</td>
<td>0.832</td>
<td>0.549</td>
<td>1.262</td>
<td>0.387</td>
</tr>
<tr>
<td>ypN2-3</td>
<td>0.608</td>
<td>0.452</td>
<td>0.818</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Effect of PMRT on overall survival by the ypN subgroups

<table>
<thead>
<tr>
<th>ypN subgroup</th>
<th>Hazard ratio</th>
<th>95%CI Low</th>
<th>95%CI High</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ypN0</td>
<td>1.325</td>
<td>0.841</td>
<td>2.087</td>
<td>0.224</td>
</tr>
<tr>
<td>ypN1</td>
<td>0.880</td>
<td>0.599</td>
<td>1.293</td>
<td>0.514</td>
</tr>
<tr>
<td>ypN2-3</td>
<td>0.685</td>
<td>0.531</td>
<td>0.885</td>
<td>0.004</td>
</tr>
</tbody>
</table>
Radiotherapy was not associated with OS among patients with ypN0 [HR: 1.325, 95% CI: 0.841–2.087, \(P=0.224\)] and ypN1 [HR: 0.880, 95% CI: 0.599–1.293, \(P=0.514\)]. There was no significant difference in DDFS with the addition of radiotherapy for all ypN subgroups.

Conclusions:
The results from this nationwide database study of breast cancer patients following modern NAC showed that PMRT did not improve survival for patients with ypN1 and ypN0. Radiotherapy might be only beneficial for ypN2-3 breast cancer patients who received NAC and mastectomy in the modern era. Randomized clinical trials are needed to optimize the use of PMRT for breast cancer patients treated with neoadjuvant chemotherapy.
Title: Prospective comparison of late toxicity and cosmetic outcome after accelerated partial breast irradiation with conformal external beam radiotherapy or single-entry multi-lumen intracavitary brachytherapy

Shane R Stecklein¹, Gildy V Babiera¹, Isabelle Bedrosian¹, Simona F Shaitelman¹, Matthew T Ballo², Welela Tereffe¹, Isidora Y Arzu¹, George H Perkins¹, Eric A Strom¹, Valerie K Reed¹, Tomas Dvorak³, Benjamin D Smith¹, Wendy A Woodward¹, Karen E Hoffman¹, Pamela J Schlembach¹, Gregory M Chronowski¹, Shalin J Shah¹, Steve M Kirsner¹, Christopher L Nelson¹, William Guerra¹, Seyyedeh S Dibaj¹ and Elizabeth S Bloom¹. ¹The University of Texas MD Anderson Cancer Center, Houston, TX; ²The University of Tennessee Health Science Center, Memphis, TN and ³UFHealth Cancer Center / Orlando Health, Orlando, FL.

Body: Purpose/Objective(s):
To prospectively compare late toxicity after accelerated partial breast irradiation (APBI) with 3D-conformal external beam radiotherapy (3D-CRT) or single-entry multi-lumen intracavitary brachytherapy.

Patients/Methods:
Two hundred eighty-one patients with pTis or pT2N0 (≤3.0 cm) breast cancer treated with segmental mastectomy were prospectively enrolled on a multi-institution observational protocol from 12/2008 – 8/2014. Patients were enrolled and treated at primary, satellite, and affiliated academic institutions. APBI was delivered using 3D-CRT or with a Contura®, MammoSite®, or SAVI® brachytherapy catheter. 3D-CRT patients were treated to 34.0 Gy (7%) or 38.5 Gy (93%) at 3.4-3.85 Gy/fx BID and brachytherapy patients were treated to 34.0 Gy at 3.4 Gy/fx BID. Per protocol, patients were clinically evaluated at 2, 6, 12, 18, and 24 months and then annually. At each clinical evaluation the radiation oncologist scored cosmetic outcome (excellent/good/fair/poor according to the Harvard Cosmesis Scale), toxicity (seroma/infection/fat necrosis/pain/telangiectasia/radiation dermatitis/hyperpigmentation/hypopigmentation/fibrosis/induration/edema/other according to CTCAE v3.0) and recurrence status.

Results:
The median age was 61 years. Of 281 patients, 211 (75%) had invasive breast cancer and 70 (25%) had in situ disease. Among patients with invasive disease, 90% were HR+/HER2-, and among patients with in situ disease, 83% were HR+. APBI was delivered with 3D-CRT in 29 (10%) patients and with single-entry multi-lumen intracavitary brachytherapy in 252 (90%) patients. Among the brachytherapy patients, APBI was delivered with the SAVI®, Contura®, and MammoSite® devices in 176 (70%), 56 (22%), and 20 (8%) patients, respectively. With a median follow-up of 49 months, rates of Grade 1 (G1) and Grade 2-3 (G2-3) toxicity are:

<table>
<thead>
<tr>
<th></th>
<th>3D-CRT</th>
<th>Brachytherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G1 (%)</td>
<td>G2-3 (%)</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>13 (46%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Fat Necrosis</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Telangiectasia</td>
<td>6 (21)%</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Seroma</td>
<td>2 (7%)</td>
<td>1 (4%)</td>
</tr>
</tbody>
</table>

Mean skin dose of the maximally-irradiated 0.1 cc (D0.1cc) of skin was significantly higher in patients who developed telangiectasia (103.4% ± 16.1% compared to 96.5% ± 18.6% of prescription dose, p=0.007) and fibrosis (100.1% ± 15.5% compared to 92.8% ± 23.0% of prescription dose, p=0.02). Crude rates of fair or poor cosmetic outcome at 2-4 and 4-6 years were 6.9% and 14.8%, respectively, for 3D-CRT and 14.8% and 21.3%, respectively, for brachytherapy (p>0.05 at both
timepoints). Five-year recurrence-free survival was 96.3% with 3D-CRT and 96.1% for brachytherapy (p>0.05).

**Conclusion:**
APBI with single-entry multi-lumen intracavitary brachytherapy is associated with increased rates of grade 1 fibrosis and seroma than APBI with 3D-CRT. Higher mean skin D0.1cc is associated with increased risk of telangiectasia and fibrosis. Despite increased low-grade fibrosis, there is no significant difference in radiation oncologist-reported fair or poor cosmetic outcome out to six years, or rate of five-year ipsilateral breast recurrence.
Meta-analysis of local recurrence of invasive breast cancer after electron intraoperative radiotherapy

Jay Harness1, Chirag Shah2, Elizabeth Brooks3, Christina Via3 and Frank Vicini4. 1St. Joseph Hospital- Center for Cancer Prevention and Treatment, Orange, CA; 2Cleveland Clinic, Cleveland, OH; 3Decision Driver Analytics / Translational Technologies International, Asheville, NC and 4Michigan Healthcare Professionals/21st Century Oncology, Farmington Hills, MI.

Body: Background: Electron intraoperative radiotherapy (IORT) can be used during breast conservation surgery to treat early-stage invasive breast cancer. While IORT may be an attractive alternative to traditional post-operative radiotherapy for many patients, its effectiveness in preventing local recurrence is still being evaluated. Using data from current clinical and observational studies, we aimed to assess the impact of single-fraction electron IORT on local recurrence rates.

Methods: Studies on single-fraction electron IORT during breast conservation surgery were identified through a search of PubMed and Google Scholar, as well as secondary referencing. Local recurrence rate was the main outcome of interest. Protocols from each publication were assessed for potential sources of heterogeneity. A meta-analysis of proportions, using binomial distribution to model the within-study variability and a random effects model, was conducted to estimate a pooled local recurrence rate. In order to estimate a 5-year recurrence rate, we applied a single-sample Poisson-normal model to model the probability of events occurring during a fixed period of time (60 months).

Results: A total of 13 independent publications were identified for abstraction. The analysis demonstrated a pooled monthly local recurrence rate of 0.02% per person-month (95% CI: 0.00 – 0.06%) for the studies with < 5 years of follow-up, 0.03% per person-month (0.02 – 0.06%) for studies with ≥ 5 years of follow-up, and 0.02% per person-month (0.01 – 0.04%) overall. Based on this model, the predicted 5-year recurrence rate is 2.7%, with a 95% confidence interval of 1.9% - 3.7%.

Conclusions: According to the published literature, the rate of breast cancer local recurrence after electron IORT was 0.02% per person-month; with an adjusted 5-year recurrence rate of 2.7%. These findings support the recent guidelines from the American Society for Radiation Oncology (ASTRO) supporting the use of electron IORT in low-risk patients.
2017 San Antonio Breast Cancer Symposium

Publication Number: P2-11-14

Title: IMRT use after lumpectomy in early-Stage breast cancer: Patterns of care and cost-consequences

Joshua A Roth¹, Jean McDougall², Lia Halasz³, Catherine Fedorenko¹, Qin Sun¹ and Shilpen Patel³. ¹Fred Hutchinson Cancer Research Center, Seattle, WA; ²University of New Mexico School of Medicine, Albuquerque, NM and ³University of Washington School of Medicine, Seattle, WA.

Body: BACKGROUND
The American Society for Radiation Oncology (ASTRO) issued a Choosing Wisely recommendation (CWr) against use of intensity modulated radiotherapy (IMRT) as part of breast conservation therapy in 2013, noting that “its routine use has not been demonstrated to provide significant clinical advantage”. Also, as IMRT is more expensive vs conventional radiotherapy (cRT), use in this setting may represent wasteful spending. The aims of this study were to characterize: 1) IMRT use immediately preceding the ASTRO CWr, 2) the cost-consequences of IMRT vs. cRT in the year after breast cancer diagnosis, and 3) excess annual U.S. national expenditure on IMRT in this setting.

METHODS
Surveillance Epidemiology and End Results (SEER) records for women age ≥66 years with a first primary diagnosis of Stage I/II breast cancer (2007-2012) were linked with Medicare claims (2007-2013). All cases had coverage for ≥12-months before/after diagnosis (or until death), and to be consistent with the CWr, had lumpectomy 1 month before to 6 months after diagnosis, and radiation therapy within 6 months of lumpectomy. We evaluated receipt of IMRT vs cRT within 1 year of diagnosis in 12 SEER registries. We also calculated mean direct medical expenditure in the year after diagnosis by summing all allowable charges from Medicare claims. Costs were inflated to 2017 USD. We evaluated differences in means by registry using ANOVA and compared mean costs for cases treated with IMRT vs cRT using t-test. We projected excess annual U.S. national expenditure on IMRT (vs. cRT) using the findings from aims 1 & 2 and assuming 209000 incident Stage I/II cases and that 55% receive lumpectomy.

RESULTS
Among 13037 women meeting all inclusion criteria, mean age was 74, 89.0% were white, and 19.8% received IMRT. Table 1 shows the proportions receiving IMRT by SEER registry and mean cost for cases treated with IMRT vs cRT. The proportion with IMRT varied significantly between registries (p<0.001), as did the mean cost for cases treated with IMRT (p<0.001) and cRT (p<0.001). Overall, cost for patients treated with IMRT was significantly higher vs those treated with cRT ($9644, p<0.001). Nationally, we estimated $219 million in annual excess medical expenditure on unnecessary IMRT vs. cRT.

Table 1: Results for Selected Registries

<table>
<thead>
<tr>
<th>Registry</th>
<th>% with IMRT</th>
<th>Mean Cost in Year After Dx ($)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IMRT</td>
<td>cRT</td>
<td></td>
</tr>
<tr>
<td>All 12 Registries</td>
<td>19.8</td>
<td>43145</td>
<td>33501</td>
</tr>
<tr>
<td>California</td>
<td>15.8</td>
<td>46245</td>
<td>36039</td>
</tr>
<tr>
<td>Detroit</td>
<td>52.1</td>
<td>41387</td>
<td>35704</td>
</tr>
<tr>
<td>Georgia</td>
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<td>40475</td>
<td>32257</td>
</tr>
<tr>
<td>Iowa</td>
<td>3.2</td>
<td>38841</td>
<td>27567</td>
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<tr>
<td>New Jersey</td>
<td>25.2</td>
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<td>35584</td>
</tr>
<tr>
<td>Seattle</td>
<td>5.8</td>
<td>40863</td>
<td>32114</td>
</tr>
</tbody>
</table>

DISCUSSION
From 2007-2013, use of IMRT in Stage I/II breast cancer was substantial (19.8%) and varied significantly across SEER registries.
Cases treated with IMRT incurred significantly higher cost ($9644) in the year following diagnosis vs. those treated with cRT. If patterns of care remain similar today, there is potentially as much as $219 million in annual U.S. national expenditure on low-value radiotherapy following lumpectomy. Our findings suggest an opportunity to improve quality in cancer care while reducing expenditure by curbing use of IMRT. Future studies should develop interventions to align practice with ASTRO CW recommendations and reduce variation in practice between regions.
2017 San Antonio Breast Cancer Symposium

Publication Number: P2-11-15

Title: Adjuvant radiotherapy of early breast cancer induces a decrease in levels of TGFβ-1, MMP-9 and PDGF

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Body: Purpose

Radiotherapy (RT) of early breast cancer is associated with cardiovascular morbidity. Transforming growth factor beta 1 (TGFβ-1) is a pro-fibrotic cytokine that also has a role in immunological activation and epithelial proliferation, but its clinical role remains unclear. Endoglin, matrix metalloproteinase 9 (MMP-9), platelet derived growth factor (PDGF), and oxidative stress are involved in regulation of TGFβ-1. 8-isoprostane is a biomarker of oxidative stress. The aim of our study was to evaluate the behavior of these fibrosis-associated biomarkers during adjuvant RT of early breast cancer.

Materials and methods

The study included 67 patients with early breast cancer or ductal carcinoma in situ (DCIS) receiving adjuvant RT, but no chemotherapy. The dose of RT was either 42.56 Gy in 2.66 Gy fractions or 50 Gy in 2 Gy fractions with or without boost of 10 or 16 Gy. TGFβ-1 (ng/ml), endoglin (ng/ml), PDGF (ng/ml), MMP-9 (ng/ml) and 8-isoprostane (pg/ml) were measured by enzyme-linked immunoassay (ELISA) from serum samples acquired before starting RT, 2 weeks (for 42.56 Gy) or 3 week (for 50 Gy) into RT and at the end of RT.

Results

Adjuvant RT induced significant decreases in the levels of TGFβ-1 (p=0.002), MMP-9 (p=0.017) and PDGF (p<0.001) from before RT to after RT (Table 1). Whereas, the levels of endoglin and 8-isoprostane remained stable. For the first 2 or 3 weeks of RT, TGFβ-1 remained stable and then decreased significantly by the end of RT (p=0.022). The decrease in MMP-9 was significant only from before RT to after RT (p=0.017). On the other hand, PDGF also decreased significantly during the first 2 or 3 weeks (p=0.016) and from 2-3 weeks to after RT (p=0.038). Although, endoglin remained stable throughout the whole RT, the decrease during first 2-3 weeks was significant (p=0.002).

Table 1 Change in biomarkers during RT

<table>
<thead>
<tr>
<th></th>
<th>Before RT</th>
<th>2-3 weeks</th>
<th>After RT</th>
<th>p¹</th>
<th>p²</th>
<th>p³</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGFβ-1, Md (IQR)</td>
<td>25.2 (21.1-30.7)</td>
<td>25.5 (21.0-31.1)</td>
<td>23.8 (19.6-26.9)</td>
<td>0.208</td>
<td>0.022</td>
<td>0.002</td>
</tr>
<tr>
<td>endoglin, Md (IQR)</td>
<td>26.7 (22.9-29.7)</td>
<td>25.3 (21.7-29.2)</td>
<td>26.5 (22.4-29.4)</td>
<td>0.002</td>
<td>0.249</td>
<td>0.098</td>
</tr>
<tr>
<td>MMP-9, Md (IQR)</td>
<td>334 (249-485)</td>
<td>330 (217-475)</td>
<td>289 (209-384)</td>
<td>0.169</td>
<td>0.167</td>
<td>0.017</td>
</tr>
<tr>
<td>PDGF, Md (IQR)</td>
<td>18.7 (13.7-23.5)</td>
<td>16.7 (13.0-22.6)</td>
<td>16.1 (12.8-19.8)</td>
<td>0.016</td>
<td>0.038</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>8-isoprostane, Md (IQR)</td>
<td>85.9 (53.0-134.3)</td>
<td>72.8 (36.2-120.5)</td>
<td>80.8 (45.7-134.3)</td>
<td>0.246</td>
<td>0.347</td>
<td>0.841</td>
</tr>
</tbody>
</table>

Md median, IQR interquartile range, ¹Wilcoxon signed ranks test for change from before RT to 2-3 weeks into RT, ²Wilcoxon signed ranks test for change from 2-3 weeks into RT to after RT, ³ Wilcoxon signed ranks test for change from before RT to after RT

Conclusion

This study demonstrates the behavior of TGFβ-1, endoglin, MMP-9, PDGF and 8-isoprostane during adjuvant RT of early breast cancer. Although the role TGFβ-1 as profibrotic cytokine is widely accepted, it has not been extensively studied in radiotherapy of breast cancer. The fibrotic effects of RT take years to manifest, including increased cardiovascular morbidity. More extensive studies, with longer follow-up, are needed to determine whether the changes in TGFβ-1 and its modulators are associated with clinical, RT related, cardiovascular complications in breast cancer patients.
The safety and pathological impact of neoadjuvant radiotherapy for local advanced breast cancer undergoing mastectomy and autologous reconstruction

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Body: Introduction

Delayed breast reconstructions are preferred if post mastectomy radiotherapy is indicated due to lower complication rates compared to immediate permanent implant or autologous reconstructions (AR) but cosmetic outcomes are inferior. Radiotherapy has a deleterious effect on implants and autologous tissue and often an interim tissue expander is place which has inherent pain and complications.

However, neoadjuvant radiotherapy (NART) prior to surgery allows for definitive oncological surgery to be performed with an immediate AR in a single operation and the avoidance of a temporary expander. The aim of this study is to assess the safety and downstaging impact of NART.

Methods

This is a prospective review of patients who underwent NART at GenesisCare Victoria, the Austin and the Alfred hospital. 59 LABC patients (median age 49.2 years) were divided into two groups; clinically staged and pathologically staged for reporting. There were 15 pathologically staged patients (pStage 2A-3C) and 43 clinically staged patients (cStage 2A-3B). All patients initially underwent NACT, followed by NART (median dose 50.4Gy in 28 fractions) to the breast, supraclavicular fossa and level 3 axilla with or without coverage of their Level 1 and 2 axilla, and/or internal mammary nodes. Approximately 6 weeks after completing NART, patients underwent definitive surgery and AR.

Results

All patients completed their NART with minimal toxicity and no break in treatment. 55 patients had a skin-sparing mastectomy (SSM) and 3 patients had a modified radical mastectomy. All clinically staged patients underwent an AD. ARs with a DIEP flap were performed in the majority of patients (51). The average length of hospitalisation was 6.2 days. The Miller Payne (MP) scoring index was used to record pathological responses in clinically staged patients. Overall 36 patients achieved significant downstaging of their disease, with MP scores of 5/5 for 20 and 4/5 for 16. Only 1 patient failed to achieve any downstaging with a MP score of 1/5. All 12 Her2 positive patients, 3/5 Triple negative patients and 5/26 Luminal A/B patients achieved a MP score of 5/5. All patients achieved R0 resection margins. This included 6 patients who had initial cT4 disease (cT4a X2, cT4b X1 and cT4d X3). 15 patients had initial cN2/3 disease and all successfully underwent their axillary dissections with R0 resections achieved. 10/15 had no involved axillary nodes with significant scarring seen in 6. 5/15 had residual involved nodes with significant scarring seen in 3 patients.

Post surgical toxicities were graded using Clavien-Dindo classification. 8 significant grade 3 toxicities were seen in 6 patients, with no grade 4 or 5 toxicities. No patients developed DVT or PE. No flap losses were seen.

Median follow up is 23 months. Cosmese was rated as good to excellent in all cases. 1 patient developed simultaneous loco-regional and distant recurrence with another 3 patients developing distant metastases only.

Conclusion

This review demonstrated that NART is a safe technique, which has not lead to an increase in surgical complication rates or resulted in a detriment in cosmetic outcome. NART can achieve a shorter, simpler reconstructive journey for patients.
Title: Is post mastectomy radiotherapy contributive in pN0-1mi breast cancers patients? Results of a French multi-centric cohort

Body: Aim: To assess the value of Post-mastectomy radiation therapy (PMRT) in breast cancer patients with no or minimal lymph nodes involvement.

Materials and methods: We retrospectively analyzed a French multi-centric cohort of 4283 patients treated between 1980 and 2013, by mastectomy and axillary dissection with or without PMRT. Practices were analyzed according 3 treatment periods (1980-1999, 2000-2005; 2006-2013). The value of PMRT on loco-regional recurrence, disease-free survival, breast cancer specific survival and overall survival was assessed in pN0-1mi patients, using multivariate analyses (logistic regression and Cox model). It was subsequently assessed according to the number of clinicopathologic recurrence-risk factors, generating a prognostic index (f-PMRT index), in an attempt to isolate a pN0-1mi patients subgroup deriving benefit from PMRT. We tested the accuracy of the Cambridge-PMRT (c-PMRT) index in the discrimination of patients with significantly different outcomes, as well as the value of PMRT in each c-PMRT prognostic group.

Results: PMRT was considered in more than half pN0-1mi patients of our cohort. Whereas matching pN0-1mi patients according to the number of clinicopathologic recurrence-risk factors led to isolate a higher-risk subpopulation (≥ 3 RR factors), PMRT had no significant impact on patients' outcomes, on multivariate analysis. Whereas the Cambridge-PMRT index had the potential to discriminate 3 patient populations with significantly different outcomes, its use did not help to the decision making for PMRT.

Conclusion: Despite a large cohort, we failed to isolate a subgroup of early breast cancer patients suitable for PMRT, in the absence of lymph node involvement.
Body: Purpose/Objectives: Radiation therapy is used by more than 50% of breast cancer patients, but radiation doses can be limited by normal tissue side effects. For example, breast cancer radiation therapy can improve breast cancer-specific survival, but increase cardiac deaths in those with left-sided cancers. Identifying genetic factors that can enhance tumor radiation sensitivity while decreasing normal tissue toxicities has the potential to improve the therapeutic ratio of radiation therapy – leading to more cures and less long-term toxicities. The use of animal models with differing genetic backgrounds to assess radiation toxicity, followed by genetic mapping of radiosensitivity phenotypes, has the potential to identify new targets that can predict cardiac toxicity from radiation therapy. This project examines how genetic host factors alter normal tissue toxicity risks from breast cancer radiation.

Materials/Methods: Inbred female SS rats and SS.BN3 consomic rats, that are genetically identical to SS rats except that chromosome 3 is inherited from the BN strain, have previously been shown to exhibit different vascular dynamics and breast tumor growth. For this study, adult female SS and SS.BN3 rats received image-guided whole heart radiation to a dose of 21 Gy (3 fields, AP and 2 laterals). Cardiac troponin was serially measured at 2, 6, and 12 weeks, and echocardiograms with strain analysis were performed at baseline and 3 months. The Student's t-test was used to compare values.

Results: The SS female rats exhibited enhanced cardiac toxicity compared to SS.BN3 rats, with cardiac troponin levels elevated at 12 weeks (0.32 ng/ml vs. 0.08 ng/ml for SS vs. SS.BN3, p=0.01), and moderate to severe pericardial effusions seen in 6 of 9 SS rats vs. 2 of 7 SS.BN3 rats. At 3 months post-radiation, echocardiograms revealed increased left ventricular posterior wall thickness at end diastole (LVPWd) in SS vs. SS.BN3 rats (0.25 vs. 0.20 cm, p=0.002) and increased left ventricular mass (LVM) in SS vs. SS.BN3 rats (1.54 vs. 1.28 g, p<0.001). Taken together, the SS female rats are more sensitive to cardiac irradiation than SS.BN3.

Conclusions: These results demonstrate that genetic variant on rat chromosome 3 alter the radiosensitivity to single fraction cardiac radiation therapy. Gene expression analysis and genetic mapping will be performed to identify the causative target(s). These models will also be expanded to test whether similar results are seen with fractionated cardiac radiation therapy. This project has the potential to enhance the effectiveness and toxicity profile of radiation therapy in breast cancer.
Title: Safety and efficacy of re-irradiation for locoregional breast cancer recurrences using pulsed reduced dose rate technique and concurrent capecitabine

Adam Burr¹ and Steven Howard¹. ¹University of Wisconsin, Madison, WI.

Body: Purpose: Locoregionally recurrent breast cancer presents a tremendous therapeutic challenge, but successful treatment can provide a durable cure. Re-irradiation has been performed infrequently in the recurrent setting due to concern for toxicity. Pulsed reduced dose rate radiation is a technique that can decrease the toxicity of re-irradiation by increasing normal tissue repair. Here, we update our previously published results of chest wall re-irradiation with an additional 16 patients and a focus on outcomes and long term toxicities.

Methods: Patients treated from 11/09/2000 to 04/21/2016 with pulsed reduced dose rate radiation therapy at the University of Wisconsin were identified by query of Aria radiation oncology record software. Patients were retreated to a median dose of 54 Gy (range 37.5-66 Gy) using pulsed reduced does rate technique, delivering radiation at an apparent dose rate of 0.067 Gy/min to allow for normal tissue repair. The median cumulative dose was 109.8 Gy (range 75 to 236 Gy). Eleven patients underwent comprehensive re-irradiation to the chest wall and locoregional lymphatics, while the remainder underwent re-treatment limited to the site of recurrence. Concurrent capecitabine was given to 15 patients, most frequently at 500mg BID (range 1000 mg to 1500mg daily). The Kaplan-Meier method was used for survival analysis.

Results: Thirty-three patients were identified who were treated with pulsed reduced dose rate radiation therapy for locoregionally recurrent invasive breast cancer with a median follow-up of 19.8 months (range 6.8-133.8 months). Sixteen patients were treated with curative intent and 17 patients were treated with palliative intent. Twenty-two patients had gross disease present at the time of treatment, 6 patients had microscopically positive surgical margins, and 5 patients were treated who had negative margins. The 2 year locoregional recurrence free survival was 70.5% by the Kaplan-Meier method for all patients and 81.5% for patients treated with curative intent. Two year overall survival was 43.6% for all patients and 72.2% in patients treated with curative intent. The rate of acute grade 3 skin toxicity was 21.2%. No other acute grade three toxicities occurred. A total of 9 patients (27.2%) developed late grade 3 or greater toxicities, including 4 patients who developed lymphedema, 4 patients who developed non-healing wounds, and one patient who developed both lymphedema and a non-healing wound.

Conclusion: Pulsed reduced dose rate radiation therapy with capecitabine is an effective method for treating patients with recurrent breast cancer. The moderate risk of toxicity is warranted in a subset of patients with high risk of disease recurrence or morbidity from disease progression. Further work, including prospective studies, is needed to determine the patients who who will benefit most from this technique.
Title: Tolerability and outcomes of radiation therapy for breast cancer in older women: A retrospective study in 817 patients

Kim I Cao¹, Flore Salviat², Alain Fourquet¹, Marie-Christine Falcou², Fatima Laki³, Philippe Beuzeboc⁴, Alexia Savignoni², Louis Bazire¹, Philip Poortmans¹ and Youlia M Kirova¹. ¹Institut Curie, Paris, France; ²Institut Curie, Paris, France; ³Institut Curie, Paris, France and ⁴Institut Curie, Paris, France.

Body: **Background:** Breast cancer (BC) management in older women requires an individual approach and is becoming increasingly topical given the aging population. Postoperative radiation therapy (RT) is a standard treatment of BC after breast-conserving-surgery in most patients but its relative benefit may be counteracted by potential side-effects, especially in elderly. The aim of this study was to assess acute and long-term radiation-induced toxicities and the impact of comorbidities on outcomes in the older women treated by RT for non-metastatic breast cancer.

**Materials and Methods:** Women aged ≥ 70 years at diagnosis, who received exclusive or postoperative RT for primary non-metastatic breast cancer, including carcinoma in situ, between 2003 and 2009 were retrieved from the Institut Curie registry. We calculated the Charlson Comorbidity Index (CCI) for each patient and collected the cardiovascular risk factors other than age (hypertension, dyslipidemia, smoking status). We analyzed overall survival (OS), progression free survival (PFS) and acute and late toxicities according to the CTCAE (Common Terminology Criteria for Adverse Events) v3.0.

**Results:** A total of 817 patients was included in this study. Median age at diagnosis was 76.6 years [70 – 93.3]. Most patients had HR+ (hormone-receptor positive) HER2- breast cancer (83.9 %). 517 patients (62.7%) had at least one cardiovascular risk factor. With a median follow-up of 6.7 years [0.5 - 13], OS at 5 years was 86.3% CI[95%][83.8 - 88.8], and PFS was 84.5% CI[95%][81.9 – 87.1]. OS at 5 years was statistically different according to the Charlson index: 90.2% CI[95%][87.2 – 93.3] for a CCI of 0, 84.6 % CI[95%][80.5 – 88.8] for a CCI of 1, and 78% CI[95%][70.5 – 86.2] for a CCI ≥ 2 (p < 0.001, log-rank test), respectively. Similar results were found for PFS (p < 0.001, log-rank test). 22.6% of patients had no toxicity; of those who experienced toxicity, most was limited to grade I or II. Only five cases (0.6%) of radiation – induced pneumonitis were reported after a median time of 16.4 months (grade I, n = 1; grade II, n = 2). One case (0.1%) of myocardial ischemia was described 14.5 months after RT. Women older than 80 years were less likely to have acute dermatitis (OR = 0.62; CI[95%][0.45 - 0.85]), long-term breast pain (OR = 0.31; CI[95%][0.14 - 0.62]), and long-term breast deformation (OR = 0.63; CI[95%][0.42 - 0.93]) compared to patients younger than 80 years. There was no significant association found between other cardiovascular risk factors and toxicities.

**Conclusion:** Radiation therapy for breast cancer in the older women is well-tolerated. An extended follow-up is planned in order to assess toxicities at a longer time horizon. Further studies could be envisaged to assess the quality-of-life during and after RT for breast cancer in the older patient population.
A prospective phase II study of TARGeted Intraoperative radioTherapy boost plus whole breast irradiation in breast cancer patients undergoing breast-conserving treatment

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Body: Background: TARGIT-A trial showed that intraoperative radiotherapy (IORT) using Intrabeam® concurrent with lumpectomy within a risk-adapted approach could be considered as an option for eligible patients with early breast cancer. To introduce IORT in clinical practice for Korean breast cancer patients, safety profiles for this new technique from clinical trial with Korean are required due to ethnic (anatomical) difference. Acute toxicity is a key hurdle for patients receiving IORT, because IORT may affect wound healing and increase wound complications. We conducted a phase II study as a feasibility test of IORT at the dose of 20 Gy for the replacement of boost-EBRT in Korean women who are candidates for breast-conserving treatment.

Methods: This single-arm trial aimed to investigate acute toxicity occurred within 6 months after IORT. In 1,119 patients receiving EBRT from TARGIT-A trial, the incidence of acute toxicity within 6 months is 15%. Compared with WBRT, we aim to prove a non-inferiority of IORT with 20 Gy. A sample size of 195 achieves 80% power to detect a non-inferiority proportion (P₀) of 0.2300 using a one-sided binomial test for non-inferiority. These results assume that the actual proportion (P₁) is 0.1500. Considering a drop-out rate of 10%, the trial would need to enroll 215 patients in total. This trial is registered with ClinicalTrials.gov, number NCT02213991.

Results: From August 2014 to November 2016, 233 women with early breast cancer were screened, and 215 undergoing IORT were enrolled. In 36 women, clinically significant complications during acute period are noted. The rate of patients experiencing acute toxicity was 16.7% (95% CI, 11.8-21.7%). The actual non-inferiority margin of our trial was 21.7% under the pre-specified margin as 23.0%. In details, There are 31 with seroma collection (more than 3 times when aspiration volume is over 10cc), 2 with wound infection, and 5 with skin break down. At a median follow-up of 17.8 months, there is no local recurrence.

Conclusions: Targeted intraoperative radiotherapy using Intrabeam® is a safe procedure for Korean breast cancer patients with acceptable toxicity profile in acute period.
Title: Adjuvant radiation is associated with decreased risk of locoregional recurrence in patients with micrometastatic invasive breast cancer

Adam R Burr¹, Claire R Brickson¹ and Bethany M Anderson¹. ¹University of Wisconsin, Madison, WI.

Body: Purpose: Determining the appropriate adjuvant treatment in patients with micrometastatic disease remains a considerable challenge in the treatment of breast cancer. Recent data suggests patients with micrometastatic disease have an intermediate risk of locoregional recurrence between patients with macrometastatic disease and patients with no evidence of axillary disease. Further work is needed to tailor treatment strategies to individual patients based on tumor characteristics. Here, we reviewed the outcomes of patients with N1mic axillary disease treated at the University of Wisconsin to assess the risk of locoregional recurrence in patients with micrometastases to determine risk factors associated with increased risk of LRR.

Methods: We identified patients with pathologic T1-T3, N1mic invasive breast cancers treated at the University of Wisconsin between 01/01/2004 and 07/01/2015. The patients were identified within the UW breast cancer registry and by query of pathologic records. We utilized the Kaplan-Meier method to determine the rate of locoregional recurrence free survival and overall survival. Multivariate analysis of patient and tumor characteristics was performed using the Cox proportional hazards model using locoregional recurrence as an outcome.

Results: 154 patients were identified with micrometastatic disease with a median follow-up of 4.1 years. The 5 year locoregional recurrence free survival and overall survival for all patients was 92.6% and 88.9%, respectively. On univariate analysisl, ER negative status (HR 8.03, p<0.001), PR negative status (HR 3.18, p =0.49), Her2 amplification (HR 5.02, p=0.005), and tumor grade (HR 2.96, p=0.005) were all significantly associated with increased risk of locoregional recurrence (LRR). Radiation therapy (HR 0.174, p=0.005) was the only therapeutic variable associated with decreased risk of LRR. Lymphovascular space invasion, close or positive margins, extranodal extension, and tumor stage were not associated with risk of LRR. Additionally, the type of axillary surgery, the type of breast surgery, the use of chemotherapy, and the use of endocrine therapy were not associated with risk of LRR. On multivariate analysis, ER negative status (HR 6.39, p=0.004), Her2 amplification (HR 5.53, p=0.006), and treatment with radiation (HR 0.14, p=0.004) remained significantly associated with risk of LRR.

Conclusion: The data presented suggest that treatment with radiation is associated with decreased LRR in patients with micrometastatic disease. Patients with Her2 amplification or ER negative status are also found to be at particularly high risk of recurrence. Future work is needed to determine strategies to decrease the rates of recurrence in these high risk groups and to define subgroups of patients with micrometastatic disease for whom radiation is particularly beneficial.
Title: Effect of postmastectomy radiotherapy on breast cancer with isolated tumor cells or micrometastases in regional lymph nodes: A propensity score matched analysis using the SEER database

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Body: Purpose: Postmastectomy radiotherapy (PMRT) has been strongly considered for patients with 1-3 positive axillary nodes (ALNs). The indications for PMRT are expanding to patients with negative ALNs but have multiple high-risk recurrence factors. However, for patients with isolated tumor cells (ITCs) in node or ALNs micrometastases, PMRT is equivocal. We aimed to determine the effects of PMRT on survival of patients with ITCs or ALNs micrometastases of breast cancer.

Methods: We identified patients with ITCs or ALNs micrometastases after mastectomy from the Surveillance, Epidemiology, and End Results database from 2004 to 2014. Overall survival (OS) and breast cancer-specific mortality (BCSM) were compared among patients after PMRT or not, using propensity score-matched analyses. Cox proportional hazards models and competing-risk models were performed in OS and BCSM analyses, respectively.

Results: We identified 11,622 eligible cases. PMRT was administered to 1,728 patients. Treatment was less frequent among patients who were older, patients with high-income, and patients with right-side tumor. T2 disease, more micrometastatic ALNs, grade 3 tumor, or HER2-negative disease increased the likelihood of PMRT. From the PMRT group, 1,728 (100%) were matched with 1,728 patients who did not underwent PMRT. In the matched dataset, OS at 5 years and 10 years were 88.1% and 74.2% in PMRT group, and were 87.8% and 77.3% in no PMRT group, respectively. Five-year and 10-year cumulative BCSM rate were 6.4% and 12.3% in PMRT group, and were 6.6% and 14.1% in no PMRT group, respectively. OS and BCSM were unaffected by PMRT after adjusting for multiple confounders (OS, hazard ratio, 0.92; 95% CI, 0.74 to 1.16; BCSM, subhazard ratio, 0.89; 95% CI, 0.67-1.18).

Conclusion: To the best of our knowledge, this is the largest study to date of the effect of radiotherapy on survival in breast cancer with ITCs or ALN micrometastases. In this population-based study, we do not find survival benefit of PMRT on patients with ITCs or ALN micrometastases.

Key words: Breast cancer, postmastectomy radiotherapy, isolated tumor cells, axillary nodes micrometastases, overall survival, breast cancer-specific mortality.
Title: Disparities in the application of post-mastectomy radiotherapy in Switzerland: A pooled analysis of 7 cancer registries over the 2003-2005 period

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Body: Background: The aim of this study was to identify factors that influence the delivery of post-mastectomy radiotherapy (PMRT) in Switzerland, and to analyze the adherence to consensus guidelines.

Methods: Based on 7 regional cancer registries covering 45% of the Swiss population, we identified 1408 women which underwent mastectomy for stage I-III breast cancer between January 1, 2003 and December 31, 2005. We categorized patients according to ASCO grouping in similar fashion to other comparable studies: low-risk group (T1/T2 N0): PMRT not routinely recommended; intermediate-risk group (T1/T2 N1): PMRT controversial; high risk group (T3-T4 and/or N2-N3): PMRT recommended. We further investigated factors leading to potential overtreatment (PMRT in low-risk group) or undertreatment (absence of PMRT in high-risk group). Data analysis was performed for the entire cohort, and separately for patients <70 years and ≥ 70 years of age. Probability of receiving PMRT was assessed using multivariable logistic regression.

Results: A total of 421 patients (29.9%) received adjuvant RT after mastectomy. The rate of PMRT delivery was 67% in the high-risk group, compared to 6% and 18% in the low-risk and intermediate-risk groups, respectively.

For patients at high-risk of chest wall recurrence after mastectomy (T3-T4 or N2-N3 disease), the risk of PMRT omission was significantly associated to older age (OR 4.25 [95% CI: 2.27-7.95] for patients ≥ 70 years) and to the absence of chemotherapy (OR 4.30 [95% CI: 1.97-9.36]). In patients with T3-T4 disease, PMRT was delivered in 77% of patients < 70 years and in 42% of patients ≥ 70 years (p<0.001). In patients with N2-N3 disease, PMRT was delivered in 82% of patients < 70 years and in 51% of patients ≥70 years (p<0.001).

PMRT was delivered to 28 patients (7%) at low-risk of recurrence after mastectomy (T1-T2 N0, negative margins). It was more frequently offered to patients <40 years of age (OR 3.86 [95% CI: 1.01-14.76]), with T2 tumors (OR 3.43 [95% CI: 1.45-8.11]) and negative hormone receptor status (OR 2.60 [95% CI: 1.04-6.50]).

Positive or close surgical margins (< 1mm) were a strong indicator for PMRT (p=0.001) and chest wall boost (p<0.03).

Conclusions: After mastectomy, one third of patients (33.26%) with high-risk disease did not receive PMRT. Even if we consider only patients < 70 years, a non-trivial proportion of patients with clear indication for treatment delivery did not receive PMRT (T3-T4 disease: 23%; N2-N3 disease: 18%). Further analyses are planned to explain the apparent failure of evidence-based guidelines to impact the adoption of PMRT in women with high-risk breast cancer.
**Title:** Real-world use and outcomes of trastuzumab for HER2+ metastatic breast cancer in Australia: Analysis of the herceptin program, 2001-2015

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**Body: Aims**
Between December 2001 and July 2015 Australian women with HER2-positive metastatic breast cancer (MBC) accessed trastuzumab in combination with taxane chemotherapy or as monotherapy via the government funded Herceptin Program (HP); we characterise their treatment patterns and survival outcomes.

**Methods**
This retrospective, whole-of-population cohort study used linked dispensing, medical services, and death records. We stratified patients into three year-of-trastuzumab-initiation groups: 1. 2001-2002 (may include patients accessing trastuzumab in later lines); 2. 2003 - October 2006 (likely first-line treated but prior to trastuzumab availability for early breast cancer (EBC)); and 3: October 2006–June 30 2015 (most representative of contemporary practice). Patients were observed until death or censored at June 30 2016.

We estimated duration of trastuzumab therapy from the initial dispensing date for MBC until 30 days after the last dispensing. We considered a gap of ≥90 days between trastuzumab dispensings a separate course of treatment. We estimated overall survival (OS) as the time from first trastuzumab dispensing until death from any cause. We used Kaplan-Meier methods to estimate the total duration of trastuzumab therapy and OS. We used dispensing dates of cancer medicines to determine concomitant treatments and used claims for echocardiography and MUGA scans to determine the timing of cardiac monitoring.

**Results**
5,895 patients accessed trastuzumab for MBC. Median age at trastuzumab initiation was 57 years (IQR: 48 – 66). 800 patients (22%) from Group 3 also received trastuzumab for EBC. Treatment details and OS are tabulated:

<table>
<thead>
<tr>
<th></th>
<th>Overall (n = 5,895)</th>
<th>Group 1 (n = 495)</th>
<th>Group 2 (n = 1,709)</th>
<th>Group 3 (n = 3,691)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median time on trastuzumab for MBC, first course, months (IQR)</strong></td>
<td>13.2 (5.7 – 26.6)</td>
<td>9.2 (3.8 – 22.5)</td>
<td>12.3 (5.4 – 23.3)</td>
<td>14.0 (6.1 – 29.8)</td>
</tr>
<tr>
<td><strong>Median OS from first trastuzumab dispensing for MBC, months (IQR)</strong></td>
<td>30.3 (13.4 – 68.7)</td>
<td>19.8 (8.9 – 38.8)</td>
<td>27.5 (12.5 – 58.9)</td>
<td>34.6 (15.1 – 82.8)</td>
</tr>
<tr>
<td><strong>Patients initiating trastuzumab, n (%):</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>monotherapy</td>
<td>1,571 (27)</td>
<td>226 (46)</td>
<td>625 (37)</td>
<td>720 (20)</td>
</tr>
<tr>
<td>+ taxane</td>
<td>3,150 (53)</td>
<td>157 (32)</td>
<td>800 (47)</td>
<td>2,193 (59)</td>
</tr>
<tr>
<td>+ hormonal therapy</td>
<td>763 (13)</td>
<td>47 (9)</td>
<td>169 (10)</td>
<td>561 (15)</td>
</tr>
<tr>
<td>+ non-taxane chemotherapy</td>
<td>376 (6)</td>
<td>65 (13)</td>
<td>115 (7)</td>
<td>217 (6)</td>
</tr>
<tr>
<td><strong>Cardiac assessment:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at baseline (60 days prior to 30 days following trastuzumab initiation)</td>
<td>3,721 (63%)</td>
<td>189 (38%)</td>
<td>831 (49%)</td>
<td>2,701 (73%)</td>
</tr>
<tr>
<td>during treatment</td>
<td>3,324 (56%)</td>
<td>150 (30%)</td>
<td>778 (46%)</td>
<td>2,396 (65%)</td>
</tr>
</tbody>
</table>
Conclusions

Our real-world estimates of OS for each patient group are both similar to and shorter than those from clinical trials published during similar time periods. Group 3 median OS is 6 months shorter than the control arm of the CLEOPATRA study (34.6 v 40.8 months) while median duration of first trastuzumab course was 4 months longer (14.0 v 10.4 months), suggesting patients continue trastuzumab beyond progression. In Group 3, 25% of patients died within 15 months of starting trastuzumab, 50% survived beyond 3 years and 25% survived beyond 7 years. These estimates will be useful for clinicians discussing expected survival time with patients in routine practice. Although the cardiotoxicity of trastuzumab is well recognised, baseline cardiac assessment was not universal, even in the most recent cohort.
Title: Is breast-conserving therapy effective in women with large ductal carcinoma in situ (DCIS) lesions? A population-based analysis

Nafisha Lalani1,2,3, Lawrence Paszat1,2,3, Sharon Nofech-Mozes2,4, Rinku Sutradhar1, Sumei Gu2, Wedad Hanna2,4, Cindy Fong3, Naomi Miller4,5, Bruce Youngson1,5, Susan J Done1,5, Alan Tuck6, Martin C Chang7, Sandip Sengupta8, Prashant A Jani9, Michel Bonin10 and Eileen Rakovitch1,2,3. 1University of Toronto, Toronto, ON, Canada; 2Sunnybrook Health Sciences Centre, Toronto, ON, Canada; 3Institute of Clinical Evaluative Sciences, Toronto, ON, Canada; 4University of Toronto, Toronto, ON, Canada; 5University Health Network, Toronto, ON, Canada; 6London Health Sciences Centre, Toronto, ON, Canada; 7Mount Sinai Hospital, Toronto, ON, Canada; 8Queen's University, Kingston, ON, Canada; 9Thunder Bay Regional Health Sciences Centre & Northern Ontario School of Medicine, Thunder Bay, ON, Canada and 10Sudbury Regional Hospital, Sudbury, ON, Canada.

Body: Background: Most women diagnosed with DCIS will be treated by breast-conserving surgery (BCS) with or without radiotherapy (RT). Data on outcomes following breast-conserving therapy are predominantly based on women with small (<25mm) lesions. The paucity of data on outcomes of women with larger (>40mm) DCIS lesions leads to uncertainty of the appropriateness of breast-conserving therapy for women with larger lesions. Specifically, it is unclear if women with large tumors experience higher risks of local recurrence (LR) and invasive LR after BCS +/- RT that would preclude recommendations of breast-conserving therapy. We report the outcomes and evaluate the impact of large tumor size (>40mm) on recurrence risk in a population of women with pure DCIS treated by BCS alone or with RT.

Methods: The cohort includes all women diagnosed with DCIS in Ontario from 1994-2003 treated with BCS +/- RT; 82% had pathology review. Treatment and outcomes were ascertained through administrative databases and validated by chart review. Cox proportional hazards model was used to evaluate the impact of tumor size (≤10mm, 11-25mm, 26-39mm, ≥40mm) on the development of any LR (DCIS or invasive) and invasive LR. The 10 and 15-year LR-free survival (LRFS) and invasive LRFS rates were calculated using the Kaplan-Meier method with differences compared using the log-rank test.

Results: The cohort includes 3262 women with DCIS treated by BCS (N=1635 had RT). Median age at diagnosis was 59 years (IQR 50-68 years). Median follow-up was 13 years (IQR 8-15 years). Distribution of tumor size: 707 (22%) ≤10mm, 524 (16%) 11-25mm, 107 (3%) 26-39mm, 84 (3%) ≥40mm, unable to determine in 1840 (56%). Women with lesions ≥40mm were more likely to be ≤50 years of age at diagnosis (p=.02), have high nuclear grade (p<.001), multifocality (p<.001), and positive margins (p<.001) compared to women with smaller lesions. On multivariable analyses adjusted for age and year of diagnosis, tumor size ≥40mm was significantly associated with an increased risk of LR compared to size ≤10mm (HR=2.5, 95%CI:1.64-3.81). Other factors associated with LR were age ≤50 years (p<.001), omission of RT (p<.001), high nuclear grade (p=.002), and multifocality (p=.0008). Tumor size ≥40mm was not significantly associated with an increased risk of invasive LR (HR=1.68, 95%CI:.94-3.04). Women with tumour size ≥40mm treated with BCS alone had lower 10 and 15 year LRFS (53% and 41%) and invasive LRFS rates (78% and 75%) compared to women with smaller lesions. However, women with larger lesions treated with RT had significantly higher LRFS and invasive LRFS rates.

Outcomes by tumour size for women with DCIS treated with BCS with or without RT

<table>
<thead>
<tr>
<th>Tumor Size</th>
<th>BCS Alone LRFS (%)</th>
<th>BCS + RT LRFS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤10mm N=707</td>
<td>10 yr 85 70 53</td>
<td>10 yr 92 86 78</td>
</tr>
<tr>
<td>11-25mm N=524</td>
<td>15 yr 79 70 53</td>
<td>15 yr 87 86 78</td>
</tr>
<tr>
<td>26-39mm N=107</td>
<td>Invasive LRFS (%)</td>
<td>0.03</td>
</tr>
<tr>
<td>≥40mm N=84</td>
<td>10 yr 84 83 75</td>
<td>15 yr 83 83 75</td>
</tr>
<tr>
<td>p-value</td>
<td>0.01</td>
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</table>

Outcomes by tumour size for women with DCIS treated with BCS with or without RT

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<tr>
<td></td>
<td>15 yr</td>
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<td>-------</td>
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</tr>
<tr>
<td>Invasive LRFS (%)</td>
<td>86</td>
<td>84</td>
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<tr>
<td></td>
<td></td>
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<tr>
<td></td>
<td>79</td>
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</tbody>
</table>

There was a significant interaction between tumor size ≥40mm and RT (p=.02).

**Conclusions:** Women with DCIS lesions ≥40mm treated by BCS alone experience significantly higher risks of LR and invasive LR compared to smaller lesions but this risk can be mitigated with the addition of RT.
Impact of the SSO-ASTRO consensus guidelines on invasive margins on the re-excision rate among patients undergoing breast conserving surgery (BCS)

Mariana Chavez-MacGregor, Xiudong Lei, Monica Morrow and Sharon H Giordano. 1The University of Texas MD Anderson Cancer Center, Houston, TX and 2Memorial Sloan Kettering Cancer Center, NY, NY.

Background: BCS has been historically associated with a high re-excision rate, driven in part by lack of consensus on what constitutes an adequate negative margin. The SSO-ASTRO consensus guideline on invasive margins defined a negative margin as no ink on tumor based on evidence suggesting that more widely clear margins do not further decrease the risk of recurrence, potentially reducing the need for re-excision. In a large nationwide cohort of breast cancer patients undergoing BCS for invasive breast cancer we evaluate the rates of re-excision following BCS before and after the SSO-ASTRO consensus guidelines were disseminated.

Methods: Breast cancer patients undergoing BCS for invasive breast cancer between January 2012 and December 2015 were identified among female beneficiaries in the MarketScan database. Patients receiving chemotherapy before surgery were excluded. Based upon presentation of the guideline recommendations in October 2013, the pre-guideline period was defined from January 2012 to September 2013. On-line publication of the guideline in February 2014 led to definition of the post-guideline period from March 2014 onwards. The peri-guideline period was defined as the time between the pre and post-guideline intervals. Any re-excision or mastectomy within 3 months of initial BCS was identified using ICD-9 or CPT codes. Overall re-excision rates and 95% CI were calculated; groups were compared using X² test. We used a regression model to evaluate the association between pre-peri-post guideline period and re-excision while adjusting for important covariates. Results are expressed as risk ratios (RRs) and 95% CI.

Results: A total of 38,573 patients were included (20,159 in the pre-guideline, 4,607 peri-guideline and 13,807 post-guideline). The overall re-excision rate was 23.9% (95% CI 23.4-24.3). The pre-guideline re-excision rate was 25.3% (95% CI 24.7-29.9) compared to 21.6% (95% CI 20.9-22.3) in the post-guideline period (p<0.001). The rate of mastectomy as the final surgical procedure was 20.2% in the pre-guideline period and 19.1% in the post-guideline (p=0.15). We observed significant geographic variability by state in the decrease of the re-excision rates. No change in re-excision rates was seen in Mississippi, Vermont, Georgia, Oregon, West Virginia, Arkansas, Oklahoma and Tennessee. An absolute decrease greater than 10% in the re-excision rate was observed in Indiana, Nebraska, Alabama, Maine and Nevada. In the multivariable analysis, patients undergoing BCS in the post-guideline period had a statistically significant decrease in the risk of re-excision compared to patients undergoing surgery in the pre-guideline period (RR=0.87; 95% CI 0.84-0.91; p<0.001).

Conclusions: There has been a statistically significant decrease in the re-excision rate after BCS associated with the time of the dissemination of the SSO-ASTRO consensus guideline on invasive margins. The wide geographical variation observed suggests differences in the adoption rates. Our study confirms the impact that guidelines have modifying patterns of practice, reducing the frequency of unnecessary surgical interventions.
Objective: Neoadjuvant chemotrapy (NACT) is the preferred option of treatment in locally advanced and select cases of early stage breast cancer, currently. One of the major aims is to downstage tumor status allowing more conservative surgery with the most acceptable cosmetic outcome. The presence or absence of residual invasive cancer after NACT, is one of the strong prognostic factors for risk of recurrence and the margin status is the other. Due to the excess degree of fibrosis after tumor shrinkage, to accurately predict margin status intraoperatively after NACT is a challenge for surgeon. The aim of the presented study is to evaluate the efficacy of continuous intraoperative ultrasound guided breast conserving surgery (IUG-BCS) in terms of margin status and re-excision rate. The relationship between intraoperative assessment of gross macroscopic and ultrasonographic margins and cavity shavings results, were also analyzed.

Methods: Between 2014 and 2017, IUG-BCS were performed to 194 patients after NACT. Surgeon performed continuous peroperative real-time sonographic margin assessment during resection, macroscopic evaluation, specimen US including sonographic analysis of six faces of each specimen, and shaved cavity margins for permanent pathologic assessment were the standard steps of our methodology.

Results: Of the 194 patients, 82 (42.5%) had pathologic complete response (pCR) after NAC. The sensitivity of intraoperative ultrasound localization was 100% (194/194 cases). Patients were on average 53 years old (range, 28-65). There was no difference with respect to patient characteristics including age, menopausal status, personal-family history, oral contraceptive usage, body mass index and tumor localization. Mean tumor size was 5.42 cm before NAC and 2.56 cm after NAC, for those excluding ypT0. Of the 112 patients without pCR, tumor free margins were obtained by means of IUG-BCS in 99% (665/672) of margins evaluated sonographically. Moreover, the involved margins were correctly identified by the surgeon via specimen sonography in %71.4 of the cases (5/7) which was confirmed by cavity shaving results. No frozen section analysis was performed and macroscopic evaluation of the specimen predicted nothing significant. According to permanent section analysis of the resected specimens and cavity shavings, no further intervention was required due to margin positivity. IUG-BCS with real-time specimen sonography were unable to predict involved margins in only two cases, both of them proved to be invasive lobular carcinoma without evidence of residual cancer on pathological examination of cavity shavings. Accordingly, negative margins were achieved in 100% of cases at the initial procedure verified by permanent analysis.

Conclusion: Continuous intraoperative ultrasound with specimen sonography is an invaluable and effective modality to achieve negative surgical margins after NACT. Furthermore, meticulous sonographic assessment of specimen margins together with cavity shavings from tumor bed could be a feasible method to decrease re-excision rates without frozen section analysis leading to cost-effectiveness. However, the accuracy of sonography should be questioned in case of lobular histology.
Title: Real-time, intraoperative detection of residual breast cancer in lumpectomy cavity margins using the LUM imaging system: Results of a feasibility study

Conor R Lanahan¹, Michele A Gadd¹, Michelle C Specht¹, Jorge Ferrer², Rong Tang¹, Upahvan Rai¹, Andrea L Merrill¹, Anna Biernacka¹, Elena Brachtel¹ and Barbara L Smith¹. ¹Massachusetts General Hospital, Boston, MA and ²Lumicell, Wellesley, MA.

Body: Background: Obtaining tumor-free margins is critical for local control in breast conserving surgery. Currently, 20-40% of lumpectomy patients have positive margins that require surgical re-excision. We assessed the LUM Imaging System for real-time, intraoperative detection of residual tumor in breast cancer patients. The LUM System has the particular advantage of assessing in vivo lumpectomy cavity walls rather than excised specimens, to enable more accurate excision of residual tumor.

Methods: Lumpectomy cavity walls of patients undergoing lumpectomy for invasive breast cancer or ductal carcinoma in situ (DCIS), were assessed intraoperatively using the LUM Imaging System (Lumicell Inc., Wellesley MA). LUM015, a cathepsin-activatable fluorescent agent, was given IV 4±2 hrs prior to surgery. Areas of fluorescence generated at potential sites of residual tumor in lumpectomy cavities were evaluated with a sterile hand-held device, displayed on a monitor, excised and correlated with histopathology.

Results: In vivo lumpectomy cavities were imaged with the LUM Imaging System in 60 breast cancer patients. 5 were imaged without dye. 55 received LUM015 dye preoperatively and were scanned intraoperatively. Median age was 60 years (range 44-79). Mean tumor size was 1.2cm (0.06-3.5cm) with 71% invasive cancers, 29% DCIS. The test set included 569 cavity margin surfaces assessed intraoperatively and excised. Image acquisition for each margin took approximately 1 second. The LUM Imaging System showed 100% sensitivity and 73% specificity for detection of tumor <2mm from the margin. Invasive ductal cancer (IDC), invasive lobular cancer (ILC) and areas of DCIS 1mm in size could be identified. 8 patients had positive margins on standard histopathology analysis (Table). The LUM System correctly identified all positive margins identified by standard histopathology and correctly predicted negative re-excisions in 2 of 8 patients. There were no serious adverse events. 1 patient had extravasation of LUM015 at her injection site with temporary blue skin staining but no other complication.

Conclusions: The LUM Imaging System allows real-time identification of residual tumor in the lumpectomy cavity of breast cancer patients. No sites of residual tumor were missed. Additional studies are underway to optimize this approach for reducing positive margins and second surgeries in breast cancer patients.

Table: Margin results in 8 patients with positive margins on initial lumpectomy specimen

<table>
<thead>
<tr>
<th>Positive lumpectomy margin histopathology</th>
<th>LUM cavity wall result (+- for tumor)</th>
<th>Tumor found at re-excision</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCIS</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>DCIS</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>DCIS</td>
<td>+</td>
<td>+ (Mastectomy)</td>
</tr>
<tr>
<td>IDC</td>
<td>+</td>
<td>+ (Mastectomy)</td>
</tr>
<tr>
<td>IDC</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>DCIS</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>DCIS</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>DCIS</td>
<td>+</td>
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</tr>
</tbody>
</table>
Title: Re-excision rates in breast conserving surgery for invasive breast cancer after neoadjuvant chemotherapy with and without the use of a radiopaque tissue transfer and X-ray system

Hans-Christian Kolberg¹, Leyla Akpolat-Basci¹, Miltiades Stephanou¹, Sarah Wetzig¹, Yüksel Cubuk², Johannes Gerharz² and Cornelia Liedtke³. ¹Marienhospital Bottrop, Klinik für Gynäkologie und Geburtshilfe, Bottrop, Germany; ²Marienhospital Bottrop, Klinik für Radiologie, Bottrop, Germany and ³Charite - Universitätsmedizin Berlin, Klinik für Gynäkologie, Berlin, Germany.

Body: Background: Published re-excision rates after breast conserving surgery for invasive breast cancer vary between 20 and 50%. In patients after neoadjuvant chemotherapy even higher re-excision rates may result from difficulties in defining the surgical target particularly in cases with excellent treatment response. Specimen radiography is reducing re-excision rates, however, defining involved margins is often difficult using standard approaches. Devices allowing horizontal and vertical examination and an exact topographic localization of the lesion in the resected tissue could reduce re-excision rates by an intraoperative detection of involved margins.

Methods: 80 patients with invasive breast cancer receiving breast conserving surgery after neoadjuvant chemotherapy and an indication for wire marking by mammography were included in this analysis. All tumors were marked with titanium clips prior to chemotherapy. In 40 patients specimen radiography was performed in a standard approach (control group), in 40 patients a tissue transfer and X-ray system based on a non-radiopaque board with radiopaque topographic markers and a stand for cranio-caudal X-rays was used (study group). A univariate analysis was carried out to evaluate the association between the use of the radiopaque tissue transfer system and the re-excision rate using a logistic regression model. Calculations were performed using the XLSTAT Biomed Software (Version 19.03, Addinsoft, NY, USA.).

Results: 19/80 patients (23.75%) required re-excision because of involved margins; among those patients, 14/40 (35%) were in the control group and 5/40 (12.5%) in the study group. The association between the use of the radiopaque tissue transfer system and the lower re-excision rate was statistically significant (p=0.023).

Conclusion: Our analysis provides a rationale for the use of a radiopaque tissue transfer system for specimen radiography in breast conserving surgery after neoadjuvant chemotherapy for invasive breast cancer in order to reduce re-excision rates. Based on these results we are planning a study including also patients receiving primary surgery.
Local recurrence of breast cancer: Salvage lumpectomy as a safe option for local treatment

Yael Sellam¹, Merav Akiva Ben David², Shira Galper², Ilanit Dromi Shahadi², Douglas Zippel², Zvi Symon² and Ilana Gelernter¹.
¹Tel Aviv University, Tel Aviv, Israel and ²Sheba Medical Center, Ramat Gan, Israel.

Body: Introduction: The best local management for breast cancer recurrence following conservative treatment for breast cancer (BC) continues to be an open question. In this study, we compared patients' outcome after salvage lumpectomy vs. patients who underwent mastectomy for ipsilateral breast tumor recurrence (IBTR).

Materials and methods: Between 1987 and 2014 we identified 121 patients with pT₀-2, N₀-3, M₀ BC who had breast conserving surgery and radiation as their primary treatment, and subsequently had IBTR (unifocal). 47 patients underwent salvage lumpectomy (SL) and 74 salvage mastectomy (SM) as the local treatment for their 1st recurrence.

Results:
Median follow-up was 14 years (2-30) from first BC diagnosis. All consecutive identified patients, 121, were included in the study. At 1st recurrence, 47 patients (39%) chose to undergo SL for their IBTR, and 74 patients (61%) opted for SM. The mean age at 1st diagnosis of BC for SL group and SM, was 52 and 47 years, respectively (p=0.006). With similar T in both groups, most patients had T1 (43%), and T2 (34%) at first diagnosis (p=0.722). The women who opted later for SM had more ALND surgery (58% vs. 33%, p=0.023) and received more neoadjuvant chemotherapy (20% vs. 4%, p=0.022) at their first BC diagnosis.

The median DFI at 1st recurrence for SL and SM group was 12 and 7 years, respectively (p=0.011). 51 local recurrences (41%) were true recurrences by location and histology. In the SL group, 78% had a documented discussion (consideration for SL vs. SM) with a surgeon/oncologist/radiation oncologist prior to surgical decision, and 70% underwent a breast MRI before final decision. Only 45% in the SM group had a discussion and 59% underwent MRI before the decision. Following surgery for their recurrence, sixteen women (34%) of SL group underwent re-irradiation (partial breast RT) and 20 (27%, p=0.04) in the SM group (chest wall scar).

For the SL and SM cohorts, 8 and 10 patients (17%, 13.5%, p=0.22) respectively, developed subsequent local recurrence as a 3rd event. The median DFI between 2nd and 3rd recurrence for SL and SM was 6.5 and 15.5 years, respectively (p=0.081). In a multivariate analysis, age at 1st diagnosis, T at 1st and 2nd recurrence, number of dissected/+ve LN, grade, type of axillary surgery as well as neo/adjuvant chemotherapy and HER2 status (both in primary and at recurrence), had no effect on 2nd recurrence occurrence for both groups; however, in MVA, undergoing SL had higher chances of having a 2nd recurrence (3rd event), p=0.020. Having re-irradiation following SL did not protect against 2nd recurrence (3rd event, p=0.42).

At a median follow-up of 14 years, 95.7% of SL patients are alive, NED, 85% are mastectomy free. 84% of patients who opted for SM are alive, NED.

Conclusions: Salvage lumpectomy following IBTR, while associated in MVA with higher second local recurrence rate than SL is not associated with inferior survival. With survival >95% at 14 years in the SL cohort, salvage lumpectomy with or without re-radiation, in a selected population (unifocal T), represents an acceptable treatment option for patients in order to delay time to mastectomy and keep the original breast without reducing BC survival. Both options should be discussed prior to any surgical decision.
Title: Rates of re-excision surgery after implementation of consensus guidelines on margins for breast-conserving surgery in stage I and II invasive breast cancer: A Nova Scotian experience

Ashley E Drohan¹ and Lucy K Helyer¹,². ¹Dalhousie University, Halifax, NS, Canada and ²IWK Health Centre, Halifax, NS, Canada.

Body: Background: In Canada, most women with early invasive breast cancer will be treated with breast-conserving surgery (BCS). The proportion of patients requiring re-excision surgery after BCS varies province to province. Until recently, there was no universally accepted definition for adequate margin width following BCS. The Society of Surgical Oncology (SSO) and American Society for Radiation Oncology (ASTRO) published consensus guidelines in 2014 to help regulate practice. The objective of this study was to determine the proportion of patients undergoing re-excision surgery before and after guideline implementation at our institution.

Methods: We performed a retrospective review of all patients with invasive breast cancer and/or ductal carcinoma in situ (DCIS) who underwent re-excision surgery between October 1, 2010- October 31, 2011 (pre-guideline) and October 1, 2014- May 30, 2016 (post-guideline). The proportion of patients requiring re-excision surgery was calculated for each time period and patient characteristics and clinical outcomes were compared.

Results: There were 188 patients identified who underwent BCS in the pre-guideline time period and 411 patients in the post-guideline time period. The proportion of patients undergoing re-excision surgery significantly decreased from 13.8 % to 7.8 % after guideline implementation (p = 0.02). Patient characteristics were similar between those who underwent re-excision before and after guideline implementation. In patients requiring re-excision surgery, histological and pathological features were similar between time periods. Margin width and indication for re-excision surgery varied between time periods. Among women who had re-excision surgery in the pre-guideline group, most (50%) had an invasive cancer margin > 1 mm. However, after guideline implementation, a frankly positive invasive cancer margin was the most common indication for re-excision surgery (65%). No significant differences in clinical outcomes were observed between patients who underwent re-excision surgery before and after guideline implementation.

Conclusion: The proportion of patients undergoing re-excision surgery after BCS has significantly decreased after SSO-ASTRO guideline implementation at our institution. This data suggests that among women with invasive breast cancer and/or DCIS, the implementation of SSO-ASTRO guidelines has led to a 43.5% decline in re-excision surgery.
Body: Introduction. In patients with advanced breast cancer, neoadjuvant chemo-therapy is performed to increase breast conserving surgery. Although MRI is known to be accurate in predicting residual cancer after treatment, if calcification remains, the issue of whether to perform surgery based on the residual tumor prediction range in mammography and MRI has not yet been solved. The objective of this study was to estimate the accuracy of residual mammographic (MMG) microcalcification and enhancing lesion in magnetic resonance imaging (MRI) in predicting residual tumor after neoadjuvant systemic treatment (NST).

Method. This is a single-center, retrospective study. We included patients with breast cancer who underwent NST and have microcalcifications in the post NST mammogram and had the surgery from January 2, 2013 to December 30, 2014. All the patients had post NST imaging exams of MMG and MRI. Final pathologic tumor size with histopathology and biomarker status was obtained after surgery. Analysis of correlation between image findings and pathology was evaluated.

Result. Of 151 patients that were included in this study, 125 patients (82.8%) had residual invasive tumor and 26 patients (17.2%) had pathologic complete response. In overall, MRI correlated better than MMG in predicting tumor size (intraclass correlation coefficient [ICC] = 0.769 vs 0.651), but for HR+/HER2- subtype, MMG had higher correlation than MRI (ICC = 0.747 vs 0.575). Specially in HR- subtype, MRI had strong correlation with pathology (ICC for HR-/HER2+ = 0.939 and TN = 0.75), while MMG tend to overestimate the tumor size (ICC for HR-/HER2+ = 0.543 and TN = 0.479).

Discussion. Overall post-NST residual microcalcifications on MMG have lower correlation with residual tumor size than MRI. In other than HR+/HER2- subtype, the extent of calcifications on pre-OP evaluation may not be accurate in evaluating the residual extent of the tumor after NST.
Title: Does breast density increase the risk of re-excision for women with breast cancer having breast conservation therapy?

Siun M Walsh¹, Sandra B Brennan², Emily Zabor³, Laura H Rosenberger¹, Michelle Stempel¹, Lizza Lebron-Zapata² and Mary L Gemignani¹. ¹Memorial Sloan Kettering Cancer Center, New York, NY; ²Memorial Sloan Kettering Cancer Center, New York, NY and ³Memorial Sloan Kettering Cancer Center, New York, NY.

Body: Background

The definition of an adequate surgical margin for breast cancer has been a hotly debated topic for over 20 years, with “no ink on tumor” now widely recognized as an adequate pathological margin for invasive carcinoma. Patients with dense breasts pose unique challenges in terms of accurate pre-operative evaluation of extent of disease and achieving adequate margins at initial surgery. The aim of this study therefore is to analyze re-excision rates and correlate with breast density and other clinical and pathological factors before and after the decision to accept 'no tumor at the inked margin” as an adequate margin.

Methods

Patients with stage I or II invasive breast cancer treated with breast conserving surgery between the 1st of June 2013 and the 31st of October 2014 were included. Patients who had surgery prior to January 1, 2014 comprise the pre-guideline group whereas those who had surgery on or after January 1, 2014 comprise the post-guideline group. Breast density was assessed by 2 independent radiologists. Inter-reader agreement was assessed using data on all study subjects and intra-reader agreement was assessed on a random sample of 121 study subjects; agreement was assessed using the kappa statistic with bootstrap confidence intervals. Logistic regression was used to model the association between breast density and re-excision, using the minimum value of breast density according to the two independent readers, within the 2 time periods. Multivariable logistic regression adjusted for patient and disease characteristics associated with re-excision on univariable analysis.

Results

The inter-reader agreement was 0.633 (95% confidence interval (CI): 0.604, 0.663) whereas the intra-reader agreement was 0.755 (95% CI: 0.663, 0.834). A total of 1205 patients were included, of whom 504 (41.8%) had surgery before the guideline change and 701 (58.2%) after. Overall 214 (17.8%) had at least one re-excision. The re-excision rate was significantly lower in the time period after the guideline change (15.1% versus 21.4%, p=0.006). There was no significant difference in tumor characteristics between the time periods. Younger age at diagnosis was the only clinicopathological factor that was significantly associated with increased breast density (p<0.001). On univariable analysis, increased breast density was associated with higher risk of re-excision (p=0.005), as was younger age, multifocality, presence of DCIS, HER2 status and extensive intraductal component (EIC). On multivariable analysis, time period, age at diagnosis, multifocality, presence of DCIS and EIC were significantly associated with re-excision, but breast density was not (OR 1.24, 95% CI 0.98-1.56, p=0.07).

Conclusions

Women who are of younger age at diagnosis are more likely to have increased breast density. Although, younger age was associated with higher rate of re-excision, we did not find breast density to be associated with a higher rate of re-excision on multivariable analysis.
Title: Does conservative surgery treatment for locally advanced breast cancer safe after neoadjuvant treatment?

Gabriela Boufelli1, Bruna Salani Mota1, Flavia Cardoso Franca1, Maira Teixeira Doria1, Jonathan Yugo Maesaka1, Marcos Desiderio Ricci1, José Roberto Morales Piato1, Fernanda Barbosa Coelho Rocha1, Aricia Helena Galvão Giribela1, Rodrigo Gonçalves1, Sergio Masili-Oku1, Max Senna Mano1, Luciano Fernandes Chala1, Bruna Maria Thompson1, Aricia Helena Galvão Giribela1, Rodrigo Gonçalves1, Sergio Masili-Oku1, Max Senna Mano1, Luciano Fernandes Chala1, Bruna Maria Thompson1, Edmund Chada Baracat1 and Jose Roberto Filassi1. 1Instituto do Câncer do Estado de São Paulo, Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil.

Body: BACKGROUND:
The aim of this study was to assess the oncological efficacy of breast conserving surgery (BCS) after neoadjuvant chemotherapy in patients with local advanced breast cancer.

PATIENTS AND METHODS:
A retrospective cohort study was conducted with locally advanced breast cancer invasive (Stage IIb to III) treated at ICESP, an oncologic referral center between 2008 and 2016. Endpoints were disease free survival (DFS), local disease free survival (LDFS) and overall survival (OS). Multivariable analyses were performed using Cox proportional hazards models.

RESULTS:
530 patients were included, 26% (138) were stage IIB, 41.9% (222) IIIA, 29.6% (157) IIIB and 2.5% (13) IIIA. 88.8% (470) were invasive ductal carcinoma. The mean age was 51.5(23-95). 95.5% and 4.5% were submitted Neoadjuvant Chemotherapy and Hormone therapy, respectively. The BCS were performed in 24.5% (130) patients versus 75.5% (400) of mastectomies. The mean follow up was 36.4(0.16-80.2) months. There were no differences in local disease free-survival 59 (95%CI 58-61) versus 60 (95%CI 57-60); p=0.4 and overall survival 56.2 (95%CI 52-60) versus 59.3(95%CI 53-65); p= 0.24 for mastectomy and BCS. The disease free survival was lower at mastectomy group 51.4 (95%CI 49-53) versus 56,8 (95%CI 53-59); p=0.01. Logistic regression models were significant only for cancer stage both patterns, although the results were better for masses, particularly when kinetic assessments were included (LR 12.8; p = 0.005)

CONCLUSION:
In our population, the BCS does not affect the overall and local disease-free survival rates, which seems to be safe to perform in patients who desire to conserve the breast after neoadjuvant treatment.
2017 San Antonio Breast Cancer Symposium

Publication Number: P2-12-12

Title: Identifying optimal candidates for three-dimensional bioabsorbable marker placement during breast cancer treatment: Incidence and predictors of postoperative complications

Benjamin C Foster¹, Theresa A Graves¹, Charu Taneja¹, Doreen L Wiggins¹, Jaroslaw T Hepel¹, David E Wazer¹ and Kara L Leonard¹. ¹Rhode Island Hospital, Providence, RI.

Body: OBJECTIVES: Radiation therapy (RT) is often an integral component of postoperative breast cancer management. Three dimensional (3D) bioabsorbable markers have been designed to assist CT-based tumor bed targeting during the RT process. There have been limited reports detailing complications following placement of such devices. This retrospective analysis attempts to identify demographic and treatment characteristics associated with complications after 3D bioabsorbable marker placement in a cohort of breast cancer patients treated at an academic medical center. METHODS: Records of 160 patients receiving a 3D bioabsorbable marker during initial breast surgery for DCIS or breast cancer were reviewed. Ten devices were removed at subsequent re-excision or mastectomy; therefore, 150 patients were ultimately evaluable. Demographic, tumor and operative/treatment characteristics were collected. Variables including body mass index (BMI), diabetes mellitus (DM), smoking, chemotherapy or RT use and excision volume (EV) were analyzed using multivariable logistic regression analysis (MVA). Endpoints included reoperation for wound complications (re-op), receipt of postoperative antibiotics (abx) and clinically palpable 3D bioabsorbable marker. RESULTS: Median follow-up was 8.2 months. Six (6/150, 4%) patients required re-op for wound complications and 5 required 3D bioabsorbable marker removal due to complications. Twenty (20/150, 13.3%) patients received abx for clinically detected postoperative wound infections. At last follow-up, 61 (61/150, 40.6%) patients noted persistent perceived fullness of the device at the lumpectomy site, and the 3D bioabsorbable marker remained palpable by the physician in 95 (95/150, 63.3%) patients. On MVA, DM and larger EV were associated with greater rates of re-op (p=0.020 and 0.012, respectively, Table 1). Mean EV was 279 cc among the re-op cohort and 85.5 cc among the no re-op cohort. DM, receipt of chemotherapy and larger EV were associated with postoperative abx prescription (p=0.005, 0.009 and 0.005, respectively, Table 2). Mean EV was 169.6 cc among those who received abx and 81.5 cc among those who did not. Larger EV was the only statistically significant predictor of a clinically palpable bioabsorbable marker during follow-up (p=0.044).

Table 1. Multivariable Analysis: Reoperation for Wound Complications

<table>
<thead>
<tr>
<th>Variable</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.986</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.020</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.999</td>
</tr>
<tr>
<td>Excision Volume</td>
<td>0.012</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>0.079</td>
</tr>
<tr>
<td>Radiation</td>
<td>0.113</td>
</tr>
</tbody>
</table>

Table 2. Multivariable Analysis: Prescription of Antibiotics

<table>
<thead>
<tr>
<th>Variable</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.571</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.005</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.099</td>
</tr>
<tr>
<td>Excision Volume</td>
<td>0.005</td>
</tr>
</tbody>
</table>
CONCLUSIONS: Rates of re-op for wound complications (4%) and postoperative infection (13.3%) were higher than expected among this cohort receiving 3D bioabsorbable markers, and were relatively high compared to historical surgical series managed without such devices. The present analysis suggests that those with larger EV, DM or receiving chemotherapy may be at greater risk for post-operative complications when a 3D bioabsorbable marker is placed. These factors should be considered when assessing candidacy for device placement.
Title: Does close margin of ductal carcinoma in situ associated with invasive breast carcinoma affect breast cancer recurrence?

Bekir Kuru¹, Savas Yuruker¹, Yurdanur Sullu¹, Bilge Gursel¹ and Necati Ozen¹. ¹Ondokuz Mayis University School of Medicine, Samsun, Turkey.

Body: Background and aims: The Society of Surgical Oncology (SSO) and the American Society for Radiation Oncology (ASTRO) consensus guidelines regarding surgical margin for patients with stage 1, 2 invasive breast cancers (IBC) associated with or without ductal carcinoma in situ (DCIS) undergoing breast conserving therapy (BCT) recommend that no ink on tumor is standard for an adequate margin and for low risk of local recurrence. However, what constitutes the adequate margin width for DCIS associated with IBC is controversial, and there are not sufficient studies on the issue. Our aim was to determine if the close surgical margin (that is, < 2 mm, but no ink on tumour) for DCIS associated with IBC leads to increased rate of local recurrence.

Material and Methods: Six hundred and twenty-eight patients with T1-2 IBC that underwent BCT and had no ink on tumour between 2009 and 2017 in our institution were included in the study. Patients who received neoadjuvant chemotherapy were excluded from the study. Age, tumour size, axillary lymph node status, resection margin status of DCIS as close than 2 mm or ≥ 2 mm, association of DCIS as present or absent, extent of extensive intraductal component (EIC) as yes or no were investigated. All patients were followed up for ipsilateral local breast recurrence.

Results: The median age was 50 (range, 29-82), and median tumor size was 25 mm (range, 5-50). Median follow up time was 46 months (range, 5-99). Of the 628 patients, 440 (70%) were found to be associated with DCIS, and 94 (15%) were associated with EIC. Of the 440 patients with DCIS, 119 (27%) had close surgical margin (<2 mm), and 321 (73%) had ≥ 2 mm surgical margin for DCIS.

Characteristics of 628 patients with invasive breast carcinoma

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Association of ductal carcinoma in situ</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>440 (70)</td>
</tr>
<tr>
<td>Absent</td>
<td>188 (30)</td>
</tr>
<tr>
<td>Resection margin status of DCIS</td>
<td></td>
</tr>
<tr>
<td>Close &lt; 2 mm</td>
<td>119 (27)</td>
</tr>
<tr>
<td>&gt;= 2 mm</td>
<td>321 (73)</td>
</tr>
<tr>
<td>Extensive intraductal component (EIC)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>94 (115)</td>
</tr>
<tr>
<td>No</td>
<td>534 (85)</td>
</tr>
</tbody>
</table>

Among 440 patients with DCIS associated with IBC, 3 local recurrences developed. One of the local recurrences developed in a patient who had close surgical margin for DCIS, and in other 2 patients surgical margins were ≥ 2 mm. One of 2 patients with surgical margin of ≥ 2 mm had also EIC.

Conclusions: No ink on tumour is adequate margin for DCIS associated with IBC in patients who underwent BCT and is not associated with increased ipsilateral breast cancer recurrence.
Title: ReFilx- synthetic biodegradable soft tissue fillers for breast conserving surgery in breast cancer

Wey Liang Leong¹,4,6, Soroor Sharifpoor³, Kyle Battiston⁷, Dan Charleton⁶, Mark Corrigan⁶, David R McCready¹,6, Susan J Done⁴,⁵ and J Paul Santerre²,3,7. ¹Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada; ²Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, ON, Canada; ³Translational Biology and Engineering Program, University of Toronto, Toronto, ON, Canada; ⁴Campbell Family Institute of Breast Cancer Research, University Health Network, Toronto, ON, Canada; ⁵Laboratory Medicine Program, University of Toronto, Toronto, ON, Canada; ⁶University of Toronto, Toronto, ON, Canada; ⁷Faculty of Dentistry, University of Toronto, Toronto, ON, Canada; ⁸Cork Breast Research Centre, Cork, Munster, Ireland and ⁹Grand River Hospital, Kitchener, ON, Canada.

Body: Introduction: Breast conserving surgery (BCS) is the most common procedure performed in breast cancers, but it can often result in breast deformities that can have negative impacts on quality of life. With better treatments, more breast cancer survivors are expected to live longer, the demand for achieving optimal cosmetic outcomes has also increased accordingly. Currently, oncoplastic techniques involving local tissue rearrangement with or without contralateral balancing procedures are used in specialized centers to achieve breast symmetry in some patients. When a breast deformity occurs, corrective options include: fat grafting, autologous flap procedures and completion mastectomy with immediate reconstruction. These techniques have long operative times, longer length of hospital stay and higher complication rates. Commercially-available synthetic implants are fabricated in pre-determined sizes and thus are not suitable to reconstruct partial breast deformities of varying size and shape. We explored the use of amino-acid based biodegradable polyurethanes as tissue fillers for BCS due to their chemical versatility, superior mechanical properties and tailored biocompatibility. Objective: To evaluate novel biodegradable polymer constructs, referred to as ReFilx, as soft tissue fillers for BCS defects. Hypothesis: Implantation of ReFilx during BCS will maintain breast shape and size and promote tissue regeneration in and around the biodegradable biomaterial, in contrast to sham controls. Methods: Two ReFilx formulations with high porosity, mechanical properties (compressive modulus=45±6 kPa and 31±9 kPa) comparable to native breast tissue and a moderate degree of swelling (202±6% and 248±6%) were selected for implantation in porcine BCS defects. Three female Yucatan Minipigs (age=4 years, weight=100-120 kg, 12 breasts per pig) received BCS to remove normal breast tissue of approximately 2 cm diameter, after which the defects were filled with ReFilx Formulation A, ReFilx Formulation B, or no filler (sham control). At 6, 12, 24, and 36 weeks post-implantation (n=3 per group), ultrasound breast examinations and mastectomies of each selected group of breasts were performed. Samples were fixed in 10% buffered formalin and stained with H&E, Masson's Trichrome and immunohistomchemistry using CD31. Results: ReFilx formulations maintained breast size and shape, with similar stiffness to native breast tissue, while sham controls collapsed over 36 weeks. The ReFilx fillers supported cell and tissue infiltration and neovascularization, as indicated by Masson's Trichrome and CD31 staining, respectively, without eliciting foreign body giant cell formation, fibrosis, or chronic inflammation, commonly associated with implanted medical devices. Conclusions: ReFilx are promising soft tissue fillers for breast volume restoration, representing a simple, versatile, permanent, and aesthetically superior solution to prevent soft tissue deformities. Acknowledgements: MaRS PoP fund, grant # MI 2011-170, NSERC # SYN 430828. Haynes Connell Foundation Breast Cancer Fund.
Title: The use of oncoplastic surgical techniques to increase successful breast conservation in invasive lobular carcinoma of the breast

Jasmine M Wong1, Merisa L Piper1, Cheryl Ewing1, Michael Alvarado1, Laura J Esserman1, Hani Sbitany1, Robert D Foster1 and Rita A Mukhtar1. 1University of California, San Francisco, San Francisco, CA.

Body: Background: Invasive lobular carcinoma (ILC) of the breast differs from invasive ductal carcinoma in its non-cohesive growth pattern. These diffuse tumors pose challenges for accurate size assessment as well as surgical resection. Patients with ILC have higher rates of positive margins and lower rates of successful breast conservation surgery. Oncoplastic surgical techniques such as oncoplastic reduction mammoplasty and lumpectomy with oncoplastic closure can allow for removal of larger areas of breast tissue than lumpectomy alone. Whether or not these techniques improve the success of breast conservation in patients with ILC is unknown.

Methods: We queried a prospectively maintained surgical database and identified 384 women treated for ILC at UCSF between 1992 and April 2017. We reviewed pathology and operative reports, and clinical outcomes data, and identified 199 women who had an initial attempt at breast conservation. Data were analyzed in Stata 14.2.

Results: Average age was 61 years (range 39-84), and 69% of patients underwent lumpectomy, 16% had lumpectomy with oncoplastic closure, and 15% had oncoplastic reduction mammoplasty. The majority of tumors were hormone receptor positive, Her2 negative, grade 2, T1 or T2, and 28% were node positive. A total of 156 women (78%) had successful breast conservation; of these, 34% had one re-excision, and 1 patient had two re-excisions. Positive margins were seen in 40% of patients overall, and were significantly lower in the lumpectomy with oncoplastic closure group and those who had shave margins taken. Among the patients who underwent lumpectomy only, obtaining shave margins was significantly associated with final negative margins (71% versus 53%, p = 0.033). Patients with oncoplastic reduction mammoplasty had significantly larger average tumor size (4.1 cm), and significantly more tissue removed (167 cm³).

<table>
<thead>
<tr>
<th></th>
<th>Lumpectomy</th>
<th>Lumpectomy with oncoplastic closure</th>
<th>Oncoplastic reduction mammoplasty</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor size (mean)</td>
<td>2.4 cm</td>
<td>2.1 cm</td>
<td>4.1 cm</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Volume tissue excised</td>
<td>65 cm³</td>
<td>83 cm³</td>
<td>167 cm³</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Positive margins</td>
<td>42%</td>
<td>19%</td>
<td>50%</td>
<td>0.022</td>
</tr>
<tr>
<td>Shave margins</td>
<td>50%</td>
<td>69%</td>
<td>79%</td>
<td>0.005</td>
</tr>
<tr>
<td>Successful breast conservation</td>
<td>75%</td>
<td>97%</td>
<td>77%</td>
<td>0.021</td>
</tr>
<tr>
<td>Re-excision rate among those with successful breast conservation</td>
<td>33%</td>
<td>26%</td>
<td>39%</td>
<td>0.571</td>
</tr>
</tbody>
</table>

Conclusions: Tailoring the surgical treatment to tumor size can increase the rate of successful breast conservation surgery for these diffuse, often non-palpable lobular cancers. For the women with the largest tumors, oncoplastic reduction mammoplasty was often recommended. This group likely represents women who were borderline candidates for breast conservation; despite this, oncoplastic reduction mammoplasty allowed 77% to ultimately have successful breast conservation. For the women with smaller tumors, removing additional tissue with shave margins and using oncoplastic techniques for closure when necessary clearly reduced positive margin rates. Surgeons should routinely obtain shave margins when performing partial mastectomy for women with ILC.
Title: Differential efficacies of DNA damaging agents in basal-like TNBC subtypes

Corena V Shaffer¹, Andrew J Robles¹, April L Risinger¹,² and Susan L Mooberry¹,². ¹University of Texas Health Science Center at San Antonio, San Antonio, TX and ²Cancer Therapy & Research Center, University of Texas Health Science Center at San Antonio, San Antonio, TX.

Body: New approaches are needed to improve long term survival for patients with metastatic triple negative breast cancer (TNBC). Women with metastatic TNBC have a worse 3-year survival compared to women with metastatic HER2+ or ER/PR+ disease.¹ Identifying the molecular vulnerabilities of TNBC subtypes could provide for optimal treatment strategies. Gene expression analyses of TNBC patient samples by Lehmann and Bauer, et al. identified 6 subtypes of TNBC.² Based on the analysis of laser dissected tumor samples, the number of tumor subtypes was reduced from 6 to 4.³ Both analyses identified two types of basal-like cancers, basal-like 1 (BL1) and basal-like 2 (BL2).²,³ The BL1 subtype is defined by the amplified expression of genes involved in cell division and DNA damage response, and RNA polymerase.² BL2 cancers express high levels of genes involved in growth factor signaling, glycolysis, and gluconeogenesis.² They show that cell lines representative of the BL1 and BL2 subtypes of TNBC are more sensitive to cisplatin than cell lines representing the other TNBC subtypes. The BL1 or BL2 categorization, however, failed to predict sensitivity to olaparib.² BL1 and BL2 representative cell lines responded differently when treated with veliparib; BL1 cells were more sensitive than BL2 cells.² We investigated the response of BL1 and BL2 cells to a variety of DNA damaging agents to determine if the BL1 or BL2 subtype was predictive of sensitivity to a particular drug. DNA damaging agents, including alkylating agents, anti-metabolites, topoisomerase inhibitors, and PARP inhibitors were tested in cells representing the BL1 and BL2 subtypes. HCC1806 cells (BL2) were found to be selectively sensitive to gemcitabine when compared to HCC1937 cells (BL1). The panel of drugs and cell lines was expanded to further understand the sensitivity of BL1 and BL2 subtypes to a variety of DNA damaging agents. Our goal is to provide preclinical evidence for the informed use of specific DNA damaging agents in the treatment of patients with BL1 or BL2 subtypes of TNBCs.

2017 San Antonio Breast Cancer Symposium

Publication Number: P2-13-02

Title: Diabetes medications and risk of breast cancer in Korean females with type 2 diabetes

Jongoh Kim¹ and Min Ji Kwak². ¹Baylor College of Medicine, Houston, TX and ²The University of Texas McGovern Medical School, Houston, TX.

Body: Background: Type 2 diabetes is associated with 20-30% increased risk of breast cancer in postmenopausal women. Several diabetes medications have been linked to increased risk of malignancy. The effect of diabetes medications on the risk of breast cancer is unknown.

Methods: We used the 2011 claim data from the National Health Insurance Service in South Korea to evaluate associations between diabetes medications and breast cancer. The data was provided as a stratified sample of the nationwide health insurance claims in 2011 without links to the previous or the following years. Clinical information was collected from sequential claims per patient for the study year. Among those who had had claims for prescription of diabetes medications or diagnosis of type 2 diabetes without diagnosis of type 1 diabetes, development of breast cancer was analyzed in association with diabetes medications using multiple logistic regression. Diabetes medications were categorized as following: metformin, insulin secretagogues (including glibenclamide, gliclazide, glimepiride, glipizide, repaglinide, nateglinide, and mitiglinide), thiazolidinediones (including rosiglitazone and pioglitazone), dipeptidyl peptidase-4 (DPP4) inhibitors (including sitagliptin, saxagliptin, and vildagliptin), alpha-glucosidase inhibitors (including acarbose and voglibose), and insulin.

Result: 52,421 female subjects with type 2 diabetes aged ≥ 50 years were included in the analysis. 3,559 (6.8%) developed breast cancer. The risk of breast cancer was not significantly different by type 2 diabetes when adjusted for age (Odds Ratio [OR]1.005, 95% Confidence Interval [CI] 0.967-1.045). Metformin was used in 44.6%, insulin secretagogues in 38.7%, thiazolidinediones in 4.5%, DPP-4 inhibitors in 9.6%, alpha-glucosidase inhibitors in 10.1%, and insulin in 2.8%. When adjusted for age and one another, OR of breast cancer was 0.819 (95% CI 0.752-0.891) for metformin, 1.050 (0.966-1.141) for insulin secretagogues, 1.605 (1.392-1.850) for thiazolidinediones, 0.781 (0.680-0.897) for DPP4 inhibitors, 0.906 (0.798-1.029) for alpha-glucosidase inhibitors, and 1.729 (1.456-2.053) for insulin, respectively

<table>
<thead>
<tr>
<th></th>
<th>unadjusted OR</th>
<th>95% CI</th>
<th>Adjusted OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metformin</td>
<td>0.826</td>
<td>0.771-0.886</td>
<td>0.819</td>
<td>0.752-0.891</td>
</tr>
<tr>
<td>Insulin secretagogues</td>
<td>0.955</td>
<td>0.891-1.025</td>
<td>1.050</td>
<td>0.966-1.141</td>
</tr>
<tr>
<td>Thiazolidinediones</td>
<td>1.571</td>
<td>1.368-1.804</td>
<td>1.605</td>
<td>1.392-1.850</td>
</tr>
<tr>
<td>DPP4 inhibitors</td>
<td>0.726</td>
<td>0.637-0.826</td>
<td>0.781</td>
<td>0.680-0.897</td>
</tr>
<tr>
<td>alpha-glucosidase inhibitors</td>
<td>0.856</td>
<td>0.760-0.965</td>
<td>0.906</td>
<td>0.798-1.029</td>
</tr>
<tr>
<td>Insulin</td>
<td>1.671</td>
<td>1.410-1.981</td>
<td>1.729</td>
<td>1.456-2.053</td>
</tr>
</tbody>
</table>

OR: odds ratio, CI: confidence interval, DPP4: dipeptidyl peptidase 4

Conclusion: Metformin and DPP4 inhibitors were associated with decreased risk of breast cancer whereas thiazolidinediones and insulin were associated with increased risk of breast cancer. The cause and effect relationship could not be established because of short duration of follow up and limited availability of confounders. But our result suggests that diabetes medications may influence the risk of breast cancer. Further research is warranted to explore the effect of different diabetes medications on the development of breast cancer.
**Title:** Statin use and breast cancer survival – a Swedish nationwide study

Signe Borgquist, Per Broberg and Håkan Olsson. 1Lund University, Lund, Sweden; 2Aarhus University, Aarhus, Denmark and 3Skåne University Hospital, Lund, Sweden.

**Body:**

**Background:** A sizeable body of evidence shows that statins can cease proliferation of breast cancer and prevent breast cancer recurrence. Given the epidemiological findings from other Scandinavian populations, we hypothesized that statins may have anticancer effects and therefore reduce cancer-related mortality in a Swedish population. This study investigates the association between both pre- and post diagnostic statin use and breast cancer outcome.

**Methods:** A Swedish nation-wide retrospective cohort study of 20,559 Swedish women diagnosed with breast cancer (July 1st, 2005 through 2008). Dispensed statin medication was identified through the Swedish Prescription Registry. Breast cancer-specific death information was obtained from the national cause-of-death registry until December 31st, 2012. Cox regression models yielded hazard ratios (HR) and 95% confidence intervals (CI) regarding associations between statin use and breast cancer-specific and overall mortality.

**Results:** During follow-up, a total of 4,678 patients died, whereof 2,669 were considered breast cancer-specific deaths. Compared to non- or irregular use, regular pre-diagnostic statin use was associated with lower risk of breast cancer-specific death (HR=0.77 (95%CI 0.63-0.95), P=0.014). When evaluating diabetes patients only (N=545), this association was strengthened (HR=0.63 (95%CI 0.40-0.98), P=0.044). Post diagnostic statin use was in similarity associated with lower risk of breast cancer-related death compared to non-use (HR=0.83 (95%CI 0.75-0.93), P=0.001).

**Conclusion:** This study supports the notion that statin use is protective regarding breast cancer-specific mortality in agreement with previous Scandinavian studies, although less so with British studies. These disparities should be investigated further.
Obesity linked genetic loci are associated with decreased risk of young onset breast cancer

Jongoh Kim¹ and Min Ji Kwak². ¹Baylor College of Medicine, Houston, TX and ²The University of Texas McGovern Medical School, Houston, TX.

**BODY:**

**BACKGROUND:** Breast cancer is the most common cancer in women. The role of several genes involved in familial breast cancer is well established. However, polygenic basis of non familial breast cancer is still not known. Obesity is known to affect risk and prognosis of breast cancer. We examined the association of genetic loci linked to obesity and breast cancer.

**METHODS:** We used the genomic microarray data (Illumina Human OmniExpressExome 8) from the Two Sister Study. The Two Sisters Study recruited young onset (<age 50 years) breast cancer patients and their sisters. We tested the association of breast cancer and 32 loci linked to obesity available from the fully processed and filtered genomic data. Conditional logistic regression was used for analysis to reflect matched pairs of breast cancer patients and control sisters. The number of minor alleles in each locus was entered into the regression model.

**RESULT:** 1,458 breast cancer patients and 525 sisters as control were included in the analysis. Mean age of diagnosis of breast cancer was 44.5 years. None of the genetic loci was significantly associated with breast cancer without matching breast cancer patients and their sisters. After matching breast cancer patients and their sisters using conditional logistic regression, rs6971091 (FAM71F1), which is linked to higher risk of obesity, was significantly associated with decreased risk of breast cancer (Odds Ratio [OR] 0.682, 95% Confidence Interval [CI] 0.488-0.953). Interestingly, one locus of FTO, rs1121980, which is also linked to higher risk of obesity, became significantly associated with decreased risk of breast cancer (OR 0.381, 95% CI 0.166-0.875) when four FTO loci (rs1121980, rs8050136, rs1421085, and rs16953002) were mutually adjusted.

**CONCLUSION:** This study showed that genetic loci linked to obesity could affect the risk of breast cancer. The two loci that we identified were linked to higher risk of obesity but associated with lower risk of young onset breast cancer. This is consistent with previous studies that showed a decreased risk of breast cancer in obese premenopausal women. Limitations of this study are that we were not able to adjust known confounders including body mass index and the number of subjects was small. However, we increased the power by incorporating data structure of sister matching to find associated genetic loci. Further research is warranted to better elucidate interaction of obesity and the development of breast cancer.
Racial differences in recurrence of hormone receptor-Positive breast cancers in the Carolina breast cancer study

Katherine E Reeder-Hayes1,2, Xuezheng Sun2,3, Andrew Olshan2,3, Lisa A Carey1,2 and Melissa A Troester2,3, 1University of North Carolina; 2University of North Carolina Lineberger Comprehensive Cancer Center and 3University of North Carolina.

Racial disparities in breast cancer outcome are most pronounced among women with hormone receptor-positive, human epidermal growth factor receptor 2-negative (HR+/HER2-) disease. Several factors may contribute to this disparity, including biologic heterogeneity within the clinical HR+/HER2- subtype, and differences in access to treatment. In this study, we leverage the unique resources of the Carolina Breast Cancer Study Phase III (CBCS-III), a large prospective cohort study including tumor bio-specimens and detailed clinical annotation from black and white women with breast cancer, to examine potential contributors to racial disparities in recurrence of HR+/HER2- breast cancer.

The CBCS-III cohort includes 2,998 women recruited between 2008 and 2013 by rapid case ascertainment from 44 counties in North Carolina, stratified by age (>50 versus <50) and race (black vs white). For this analysis, we included 1,629 women with stage I-III, HR+/HER2- clinical phenotype. We performed Cox proportional hazards regression to assess the effect of race on recurrence-free survival (RFS). Model 1 was minimally adjusted for age. Model 2 additionally adjusted for clinical factors likely to vary by race and to affect recurrence risk, including stage, grade, and the receipt of adjuvant chemotherapy. Finally, for patients who had specimens available (n=935), models were fit adjusting for clinical variables as well as PAM50 Risk of Recurrence score with both proliferation index and tumor size (ROR-PT) (Model 3) or molecular subtype dichotomized as Luminal A versus other (Model 4).

With a median follow-up time of 5.5 years, 8.6% (118/1371) of whites and 14% (196/1403) among blacks had recurrence. After adjustment for age, black patients were at significantly higher risk for recurrence (HR=2.01, 95% CI 1.40-2.88), and this disparity was only partially attenuated by adjusting for baseline clinical factors (HR 1.62, 95% CI 1.11-2.36). Additional adjustment for molecular features by either ROR-PT score or molecular subtype further attenuated, but did not eliminate, the disparity in recurrence risk (HR=1.51, 95% CI 0.81,2.81 using ROR-PT and HR=1.44, 95% CI 0.77-2.69 using subtype category). Results are summarized in Table 1.

| Models of Recurrence Free Survival by Race (white = ref) |
|-----------------|-----------------|
|                 | HR              | 95% CI          |
| Model 1         | 2.01            | (1.40, 2.88)    |
| Model 2         | 1.62            | (1.11, 2.36)    |
| Model 3         | 1.51            | (0.81, 2.81)    |
| Model 4         | 1.44            | (0.77, 2.69)    |

Adjusted for: (1) age; (2)age, AJCC stage, grade, chemo; (3)ROR-PT, assay technique factor, and variables in Model 2; (4)PAM50 subtype, assay technique factor, and variables in Model 2.

In this recent population-based cohort, black women with HR+/HER2 negative disease remain twice as likely as their white counterparts to experience a recurrence within 5 years. Clinical features at disease presentation do not fully explain these outcome differences, while adjustment for biologic heterogeneity by either ROR-PT score or molecular subtype attenuates the observed disparity more than clinical features alone. There are likely residual factors not measured in this analysis, such as endocrine therapy under-use or other biologic differences, which may be targetable determinants of survival disparities. These hypotheses are being actively explored in CBCS-III.
2017 San Antonio Breast Cancer Symposium

Publication Number: P2-13-06

Title: Pancreatic nutrition program (PNP): A novel weight reduction program for breast cancer survivors

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Body: BACKGROUND: Breast cancer survivors have a high prevalence of metabolic dysfunction—characterized by high glucose and weight gain. Regardless of menopausal status, overweight and obese women are at increased risk for developing breast cancer and those who are diagnosed with breast cancer experience adverse cancer-related outcomes. The underlying principle of the Pancreatic Nutrition Program (PNP) is that bio-individualized healthy food choices—eating the correct foods and food combinations for an individual's body—can minimize fluctuations in insulin by keeping blood glucose regulated (70-100 mg/dL) and this will promote sustained weight loss, improved health, and quality of life. METHODS: The primary endpoint was change in body weight at 24 weeks post-PNP. The study was powered to detect a 10% loss of weight from baseline. Secondary endpoints included change in: glucose levels, insulin resistance, body composition, body chemistry, physical fitness, biological markers, quality of life, and compliance. Postmenopausal, non-diabetic breast cancer survivors (stages I-III) within 5 years of completion of treatment who had a body mass index of 25-33 kg/m² were recruited. For the first 12 weeks, patients wore a glucometer (Abbott), which recorded glucose every 15 minutes continuously, and kept a food journal. During weekly meetings, glucometer data was reviewed with journal entries to identify food choices and combinations that would kept the subject's glucose levels between 70-100 mg/dL. At the end of the 12-weeks, the weekly meetings and glucometer were discontinued and patients were expected to maintain the PNP for an additional 12 weeks. Study endpoints were measured at baseline, 12-week and 24-week visits. RESULTS: Of the 21 patients enrolled in the study, 12 were non-Hispanic Caucasian, 5 were Hispanic, 2 were African-American, and 2 were Asian. The median age was 56 years (43-76 years). Twenty were estrogen-receptor positive, 18 progesterone-receptor positive, and 8 were HER2/neu positive. The mean body weight at baseline was 170.9 lbs (±20.4 lbs). Two patients dropped out prior to 12-weeks and 1 developed recurrent disease. Among the 18 eligible women who completed the first 12 weeks, the median weight loss at 12-weeks was 10.1 lbs (1.5-19.6 lbs). The median waist circumference lost was 2.5 inches (gain of 0.4 inches-loss of 5.5 inches). Among the women whose total cholesterol was above 200 mg/dL, 71% reduced their cholesterol below 200 mg/dL by 12-weeks. All women who had triglyceride levels above 150 mg/dL reduced their levels below 150 mg/dL by 12-weeks. Likewise, among women who were identified as being pre-diabetic based on fasting glucose or hemoglobin A1c levels, all were within normal range at 12-weeks. 6-month testing will be completed in August. Among the 15 women eligible for 6-month testing, 8 (53%) completed the testing. Of those, 7 (88%) maintained their positive results. CONCLUSIONS: Bio-individualized food choices based on glucose response combined with culturally-sensitive nutrition counseling may provide a feasible mechanism for sustainable weight loss in a population at high-risk of metabolic dysfunction. However, to increase adherence, a tapering strategy should be developed after the first 12-weeks of health counseling.
Body: Background
Metaplastic breast cancer (MBC), characterized by a mixture of epithelial, squamous or mesenchymal elements and a usually triple-negative (TN) phenotype, accounts for <1% of breast cancer diagnoses. MBC has a poor prognosis with frequent distant spread, but, paradoxically, a lower than expected rate of nodal positivity (6 to 40%). Due to its rarity there is little data on how best to evaluate and manage the axilla in women with these tumors. Thus we undertook this study to evaluate axillary management and oncologic outcomes.

Methods
With IRB approval, we identified adult patients diagnosed with MBC at our institution from 2001 to 2011 from our prospective surgical pathology database. Patient, pathology, imaging, treatment and outcome data were obtained from electronic medical record, tumor registry, pathology slide and imaging review. Median follow-up for surviving patients was 66 months. Statistical analyses were performed using JMP 10.0 software.

Results
We identified 41 MBC patients, median age 60 years (range 33-89 years), with a median tumor size of 2.7 cm; 33 (80%) were TN. 23 patients (56%) had a preoperative axillary ultrasound (AUS): 9 (39%) showed at least one suspicious axillary lymph node (LN) of whom 6 had a preoperative fine needle LN biopsy (FNA) of which 3 were positive for metastasis. 6 patients, including the 3 LN+ on FNA, had neoadjuvant chemotherapy (NAC). Operation included axillary dissection (ALND) in 14, sentinel LN biopsy (SLNB) in 23, and SLNB followed by ALND in 1, while 3 patients had no axillary surgery. 10 patients were LN+ at operation. Among 22 patients who had both an AUS and axillary surgery, AUS had a sensitivity of 100% and specificity of 78%. Patient and tumor variables in association with pathologic LN status are summarized in the table. LN positivity correlated with increasing tumor size, T stage, grade and angiolymphatic invasion. 16 patients recurred, most with distant disease (10/16, 63%), although there was a solitary axillary recurrence 8 months after a negative SLNB in one patient who did not have a preoperative AUS. Thus the accuracy of SLNB was 96% (23/24) overall, but among those without preoperative AUS, 1/7 (14%) SLNBs were falsely negative. 5-year disease-free and breast cancer-specific survival estimates were 49% and 63%.

<table>
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<th>p-value</th>
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**Dominant Histology**

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*any squamous component

**Conclusion**

Our study is the first to specifically address AUS and SLNB for patients with metaplastic breast cancer. AUS had 100% sensitivity and 78% specificity, while one patient without a preoperative AUS had a falsely negative SLNB. Further, AUS with FNA of suspicious LNs was useful for staging at the time of diagnosis and informing treatment. We recommend this approach for patients with MBC.
Title: Overview of the pathological results and treatment characteristics in the first 1000 patients randomized in the SERC trial: Axillary dissection versus no axillary dissection in patients with involved sentinel node

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Body: Background: Three randomized trials have concluded at non inferiority of omission of complementary axillary lymph node dissection (cALND) for patients with involved sentinel node (SN). However, we can outline strong limitations of these trials to validate this attitude with a high scientific level. We designed the SERC randomized trial to compare outcomes in patients with SN involvement treated with ALND or no further axillary treatment. The aim of this study was to analyze results of the first 1000 patients included.

Patients and Methods: SERC trial is a multicenter non-inferiority phase 3 trial. Multivariate logistic regression analysis was used to identify independent factors associated with adjuvant chemotherapy administration and non-sentinel node (NSN) involvement. Results: Of the 963 patients included in the analysis set, 478 were randomized to receive cALND and 485 SLNB alone. All patient demographics and tumor characteristics were balanced between the two arms. SN ITC was present in 6.3% patients (57/903), micro metastases in 33.0% (298), macro metastases in 60.7% (548) and 289 (34.2%) were non eligible to Z0011 trial criteria.

Whole breast or chest wall irradiation was delivered in 95.9% (896/934) of patients, adjuvant chemotherapy in 69.5% (644/926), endocrine therapy in 89.6% (673/751) and the proportions were similar in the two arms. The overall rate of positive NSN was 19% (84/442) for patients with cALND. Crude rates of positive NSN according to SN status were 4.5% for ITC (1/22), 9.5% for macro metastases (13/137), 23.9% for macro metastases (61/255) and were respectively 29.36% (64/218), 9.33% (7/75) and 7.94% (10/126) when chemotherapy was administered after cALND, before cALND and for patients without chemotherapy.

Conclusion: The main objective of SERC trial is to demonstrate non inferiority of cALND omission. A strong interaction between timing of cALND and chemotherapy with positive NSN rate was observed.
Title: The loss of lymph node metastasis after neoadjuvant chemotherapy in patients with cytologically proven node-positive primary breast cancer

Maki Namura, Naoki Hayashi, Hiroko Tsunoda, Atsushi Yoshida, Junko Takei, Koyu Suzuki, Seigo Nakamura and Hideko Yamauchi. 1 St. Luke’s International Hospital and 2 Showa University, School of Medicine.

Body: Purpose: While the sensitivity to neoadjuvant chemotherapy (NAC) depends on breast cancer subtype, it has been reported that over 30% of patients with node-positive breast cancer achieved an axillary pathologic complete response (pCR) after NAC. However, axillary lymph node dissection (ALND) still remains as a standard treatment because of the difficulty of assessment of lymph node (LN) status after NAC. ALND will be omitted if axillary LN status is accurately assessed. Our purpose of this study was to predict the loss of axillary LN metastasis after NAC in primary breast cancer patients.

Patients and Methods: Among 997 consecutive patients who underwent surgery after NAC from January 2006, to December 2016, 279 patients with cytologically proven node-positive were included in this analysis. All patients were assessed using CT or PET-CT, and ultrasonography (US) before NAC. LN status after NAC was assessed by US. Patients with cT4 tumor, and supra/subclavicular and parasternal LN metastasis were excluded. Clinical LN status after NAC (ycN) was compared to pathological LN status (ypN) on surgical specimen. The association between LN status and clinicopathological factors including nuclear grade (NG), tumor size, the use of trastuzumab, and breast cancer subtypes, was assessed.

Result: Of the 279 patients with LN-positive before NAC, 166 patients (59.5%) had ER+/HER2- tumor, 51 patients (18.3%) had ER+/HER2+ tumor, 33 patients (11.8%) had ER-/HER2- tumor, and 29 patients (10.4%) had ER-/HER2+ tumor. 179 patients (64.2%) had ycN0 and 102 patients (36.6%) had ypN0. There was significant difference of rate of the loss of LN metastasis; 37 of 166 patients (22.3%) with ER+/HER2- tumor, 24 of 51 patients (47.1%) with ER+/HER2+ tumor, 19 of 33 patients (57.6%) with ER-/HER2- tumor, and 22 of 29 patients (75.9%) with ER-/HER2+ tumor, (p<0.01). The accuracy of assessment of the loss of LN metastasis by US (ycN0/ypN0) was high in 20 of 25 patients (80.0%) with ER-/HER2+ tumor and in 14 of 19 patients (73.4%) ER-/HER2- tumor compared to ER+ tumor; 21 of 39 patients (53.8%) with ER+/HER2+ tumor and 34 of 96 patients (35.4%) with ER+/HER2- tumor (p<0.01). For patients with ycN0/ypN+, the median number of residual LN metastasis was 1 in ER-/HER2+ tumor (range:1-2) and ER-/HER2- tumor (range:1-3), and 2 in ER+/HER2+ tumor (range:1-6) and ER+/HER2- tumor (range:1-14). Among patients with ER-/HER2+ tumor, there was association between the loss of LN metastasis and the use of trastuzumab (p<0.01). There was no association between the loss of LN metastasis and NG or tumor size.

Conclusion: Our results showed patients with ER-/HER2+ tumor and cytologically proven LN metastasis who received NAC with trastuzumab might have the loss of LN metastasis if assessed as ycN0 by US after NAC, whereas, the patients in ER+ tumor have a high risk to have residual LN metastasis after NAC even if assessed as ycN0. Further studies are warranted the prognostic impact of the omission of ALND for these populations.
**Title:** The axillary lymph node to primary breast tumor SUV ratio on FDG-PET/CT in FDG avid primary breast cancers: Could predict the necessity for axillary lymph node dissection

Min Kyoon Kim¹, Han Kyul Shin¹ and Hee-Chul Shin¹. ¹Chung-Ang University Hospital, Seoul, Korea.

**Body:**

**Background:** Emerging evidence has indicated that breast cancer patients with a low axillary burden do not benefit from sentinel lymph node biopsy. Thus, to specifically identify more than 3 nodes-positive patients who can proceed directly to axillary lymph node(ALN) dissection, and avoid unnecessary surgical procedures, accurate preoperative detection by radiologic assessment would be anticipated. In this study, we evaluated the usefulness of ALN to primary breast tumor SUV ratio (determined by \(^{18}\)F-FDG PET/CT) and MRI for predicting the need for ALN dissection in breast cancer surgery.

**Method:** Three hundred sixteen consecutive female patients with primary breast cancer were enrolled in this retrospective study between January 2012 and December 2016. All patients underwent preoperative \(^{18}\)F-FDG PET/CT, MRI, and surgical resection without neoadjuvant chemotherapy. The ALN to primary breast tumor SUV ratios(LN/T ratios) were calculated, and optimal cutoff values were determined by receiver operating characteristic curve analysis for predicting the presence of \(\geq 3\) ALN metastasis. The diagnostic performances of \(^{18}\)F-FDG PET/CT \(_{LN/T}\) ratio and MRI for the prediction of \(\geq 3\) ALN metastasis were determined by sensitivity, specificity, and diagnostic odds ratio(DOR). Subgroup analysis of those for FDG avid cancers were performed.

**Result:** Of the 316 patients, 36(11.4%) showed involvement of \(\geq 3\) ALNs, and 101(32%) had one or more metastatic lymph nodes. The mean SUV of the primary tumor in the 316 patients was 3.9, ranging from 0 to 26.6, while the mean SUV of the ALN was 0.81, ranging from 0 to 21.9. Axillary \(^{18}\)F-FDG uptake was positive in 75(23.7%) patients and optimal criteria of LN/T ratio for detecting the needs for ALN dissection was 0.3 determined by ROC analysis. MRI showed findings of suspicious ALN involvement in 147(46.6%) patients. The sensitivity and specificity of MRI were 0.89 and 0.56, while those of PET/CT \(_{LN/T}\) ratio were 0.69 and 0.87. In the receiver operating characteristic(ROC) analysis, the area under the curve(AUC) for MRI and PET/CT \(_{LN/T}\) ratio was 0.756 (0.682-0.829, 95% confidence interval), and 0.817(0.733-0.900, 95% confidence interval). Further analysis of the DOR for MRI showed a value of 10.37 and for PET/CT \(_{LN/T}\) ratio the DOR was 9.7. But, in a subgroup of patients with FDG-avid primary tumor(FDG above 3.9, n=108), the area under the curve was improved to 0.896 (0.817-0.975, 95% confidence interval) for PET/CT \(_{LN/T}\) ratio, while those of MRI was worsened. (0.681, 0.569- 0.793., 95% CI) DOR value of PET/CT \(_{LN/T}\) ratio for FDG avid cancers was 25.68 and their sensitivity and specificity were 0.83 and 0.84 each.

**Conclusion:** In FDG avid primary breast cancer, PET/CT \(_{LN/T}\) ratio could predict need for ALN dissection with higher accuracy than MRI. PET/CT has high potential for being used as a non-invasive imaging diagnostic technique identifying \(\geq 3\) ALNs metastases.
Title: A feasibility study of sentinel lymph node detection and analysis of safety to omit axillary lymph node dissection in clinically node-negative breast cancer patients after neoadjuvant chemotherapy

Kazuma Maeno, Kana Yamamoto, Mayu Ono, Takaaki Oba, Asumi Iesato, Koichi Ono, Tokiko Ito, Toshiharu Kanai and Ken-Ichi Ito. Shinshu University School of Medicine, Matsumoto, Nagano, Japan.

Body: Background: Concerns still remain about lower sentinel node (SN) detection and higher false-negative rates (FNR) in breast cancer patients treated with neoadjuvant chemotherapy (NAC), especially those who are clinically node-positive before NAC. It is necessary to analyze the validity of sentinel node biopsy (SNB) after NAC and evaluate whether the SN identification rate and FNR for clinically N0 (cN0) patients after NAC would be acceptable or not in order to omit axillary lymph node dissection (ALND).

Objectives and methods: We identified SN by radioisotopic methods followed by completion of ALND in cN0 patients after NAC from 2013 to 2016 as part of a clinical research study (SNB group, N=68) to analyze the accuracy of SNB, and retrospectively investigated the prognosis of patients treated with NAC from 2006 to 2012 (control group, N=92) to evaluate whether the validity of SNB would be acceptable or not.

Results: Mean patient ages in the SNB group and control group were 51.0 years and 49.5 years, respectively (p=0.17), and the distribution of intrinsic subtypes was not significantly different between the two groups. The numbers of cN1≤ before NAC in the SNB group and control group were 85.5% (57/68) and 80.4% (74/92), respectively (p=0.58), and the pathological complete response rates were 25.0% (17/68) and 19.6% (18/92), respectively (p=0.41). Lymphoscintigraphy using 99mTc-phytate acid was performed in the SNB group, and hot spots were detected at the ipsilateral axilla in 62 of 68 (91.2%) patients, in all of whom SNs could be identified by using a gamma-probe. The FNR, which indicates no metastasis in SNs and metastasis in non-SN, was 5.9% (4/68). Among these four patients, three were of the luminal type while one was triple negative. The number of patients without metastasis in both SN and non-SN, which are candidates for omission of ALND, was 26 of 68 (38.2%). They included eight of nine HER2-enriched patients and six of 13 luminal-HER2 patients. Conversely, there were no false-negative cases in these subtypes. Moreover, all SNs were identified even if there were metastases in SNs. Regarding the prognosis of the control group, the 10-year disease free survival of post-NAC N0 (ypN0) (52/92, 56.5%) and ypN1≤ (40/92, 43.5%) were 80.7% and 61.2%, respectively (p=0.08); in addition, the 10-year overall survival of yN0 and ypN1≤ were 90.4% and 72.6% (p=0.26). Thus, the prognosis of ypN1≤ was not significantly inferior to that of ypN0. If omission of ALND were performed for false negative cases, then the risk of axillary relapse would be a concern. However, these data indicate the possibility that 5.9% of FNR as shown in the SNB group would not have much influence on prognosis.

Conclusion: The accuracy of SN detection by radioisotopic methods for cN0 breast cancer after NAC was not maintained like that for -early breast cancer although it was better than the results of previous studies. However, there were no false-negative cases in HER2-enriched and luminal-HER2 subtypes, which could be potential candidates for omission of ALND. In addition, omission of ALND for false-negative patients would have less influence on the prognosis.
**2017 San Antonio Breast Cancer Symposium**

**Publication Number:** P3-01-06

**Title:** Can axillary evaluation be omitted in patients preoperatively diagnosed with ductal carcinoma in situ by core needle biopsy?

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**Body:**

**Background**

Patients diagnosed with ductal carcinoma in situ (DCIS) by core needle biopsy (CNB) have a great chance of upgrading invasive cancer on the final pathology. Positive axillary lymph nodes can be found in these patients. The present study sought to identify clinicopathological factors associated with upgrading and axillary lymph nodes metastasis in patients preoperatively diagnosed with DCIS by CNB.

**Materials and Methods**

This study identified 604 patients (cT1-3N0M0) with preoperative diagnosis of pure DCIS by CNB who had undergone axillary evaluation from August 2006 to December 2015 at Fudan University Shanghai Cancer Center (FUSCC). Predictors of upgrading and axillary lymph nodes metastasis were analyzed, respectively.

**Results**

Of all 604 patients, 513 (84.93%) and 91 (15.07%) patients had undergone sentinel lymph nodes biopsy (SLNB) and axillary lymph nodes dissection (ALND), respectively. Overall, 121 (20.03%) and 193 (31.95%) patients were upgraded to DCIS with microinvasion (DCISM) and IDC on final pathology, respectively. Positive axillary lymph nodes were identified in 41 (6.79%) patients, of which 35 (5.80%) patients had 1-2 positive axillary lymph nodes, 6 (0.99%) patients had 3 or more positive axillary lymph nodes. Among patients with axillary lymph nodes metastasis, 4 (9.76%), 4 (9.76%) and 33 (80.48%) patients were in DCIS, DCISM and IDC group, respectively. Predictors of upgrading included tumor size on ultrasonography ($P=0.001$), Ki-67 ($P=0.046$) and molecular subtype ($P=0.007$) in univariate analysis. In multivariate analysis, patients with larger tumor size on ultrasonography (>2cm) (OR 1.767, $P=0.001$) were more likely to be upgraded on final pathology. Also, ER+ HER2+ patients were more likely to be upgraded than ER+ HER2- patients (OR 1.659, $P=0.047$). Factors associated with axillary lymph nodes metastasis included nipple discharge ($P<0.001$), tumor size on pathology ($P=0.037$), number of lesions ($P=0.039$), axillary evaluation methods ($P=0.029$) and molecular subtype ($P=0.049$) in univariate analysis. Whereas, only nipple discharge and larger tumor size on pathology (>2cm) reached statistical significance in multivariate analysis (OR 5.959, $P<0.001$; OR 2.361, $P=0.042$).

In addition, further analysis showed upgrading on final pathology had a significant influence on axillary lymph nodes status ($P<0.001$). However, this correlation was not shown between patients with DCIS and DCISM in pairwise comparison.

**Conclusion**

The data of upgrading and axillary lymph nodes metastasis in patients with an initial diagnosis of DCIS by CNB was comparable in this cohort with published data. Despite of a 51.98% upgrading rate, the rate of axillary lymph nodes metastasis in these patients is low, which supports the omission of axillary evaluation in selected patients.
Comparison of the prognostic value of lymph node ratio versus residual lymph node status in triple negative breast cancer

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Body: Background: Triple negative breast cancer (TNBC) patients with residual disease are characterized for a bad outcome. In these patients nodal involvement after neoadjuvant chemotherapy (NAC) is one of the most important prognostic determinants. In this study we compare two prognostic variables based on nodal status, the lymph node ratio (LNR=number of positive nodes/ number of resected nodes) and residual lymph node status (ypN) in TNBC who received NAC.

Methods: We evaluated a retrospective cohort of TNBC patients with residual disease in the lymph nodes (at least 4 resected nodes) and treated with NAC between 2000-2014 at the Instituto Nacional de Enfermedades Neoplasicas (Lima-Peru). Patients were grouped in three LNR groups (≤0.20, 0.20-0.69 and ≥0.70). Survival differences were calculated by the log rank test. Prognostic factors for progression free survival (PFS) and overall survival (OS) were assessed by the Cox regression analysis.

Results: Overall, 189 were evaluated. Mean age was 48y (range: 26-73) where 53.5% were premenopausal and 16.5% had breast/ovary cancer familial background. A total of 175 pts (92.6%) had clinical T3-T4 tumors and 85.2% had nodal involvement at diagnosis. Indeed, the majority of patients had III CS (94.2%). Regarding to the LNR, 48.2% (n=91), 29.6% (n=56) and 22.2% (n=42) (22.2) had LNR ≤0.20, 0.21-0.69 and ≥0.70, respectively. Distribution of clinical differences was similar between groups, except for the clinical N stage (N2-N3: 15.4%, 46.4%, 52.3%, respectively; p<0.001). The median follow-up was 7 years. Progression risk was higher in patients with LNR ≤0.20 than 0.20-0.69 and ≥0.70 (HR=1.77, 95%CI:1.21-2.59, p=0.003 and HR=2.22, 95%CI:1.47-3.35, respectively, p<0.001). It was similar for the risk of death (HR=1.78, 95%CI: 1.17-2.70, p=0.007 and HR=2.95, 95%CI:1.91-4.56, respectively, p<0.001). LNR groups were associated to progression events (P=0.02) in contrast to ypN groups (P=0.07). In the multivariate analysis, pre-menopausal status, a higher LNR and ypT with non-complete response were prognostic factors of worse DFS. Only a higher LNR has a negative impact on OS (table 1).

Conclusion: LNR was an independent prognostic factor for TNBC in patients with residual disease with better capability than ypN to predict progression events. LNR should be considered in the risk stratification after NAC among these patients.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>DFS</th>
<th>OS</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95%CI</td>
</tr>
<tr>
<td>Premenopausal status</td>
<td>1.93</td>
<td>1.37-2.73</td>
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<td>LNR 0.20 vs 0.21-0.69</td>
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<td>1.36-2.97</td>
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<tr>
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<td>1.57-3.74</td>
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<td>ypT complete vs partial</td>
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<tr>
<td>ypT complete vs non-response</td>
<td>2.04</td>
<td>1.20-3.48</td>
</tr>
</tbody>
</table>

ns=not significant
Title: Axillary staging after neoadjuvant chemotherapy: The comparison of surgeon performed axillary ultrasound and 18F-FDG PET/CT with pathologic status of sentinel lymph nodes in clinically node-negative breast cancer

Guldeniz Karadeniz Cakmak¹, Rabiye Uslu², Ali U Emre¹, Ozlem Elmas³, Yucel Karadere¹, Mine Buse Konuk¹, Huseyin Engin⁴ and Banu Dogan Gun⁵. ¹BEUN The School of Medicine, Zonguldak, Turkey; ²BEUN The School of Medicine, Zonguldak, Turkey; ³BEUN The School of Medicine, Zonguldak, Turkey; ⁴BEUN The School of Medicine, Zonguldak, Turkey and ⁵BEUN The School of Medicine, Zonguldak, Turkey.

Body: Objective: In the era of neoadjuvant chemotherapy (NAC), the most accurate method for axillary staging is a challenge for surgeon. Sentinel lymph node biopsy (SLNB) is the recommended choice of care for axillary staging in clinically node-negative (cN0) disease. Nevertheless, the role of preoperative axillary ultrasound (AUS) or 18F-FDG PET/CT in case of cN0 patients after NAC (ycN0) is controversial. The purpose of the presented study is to assess the correlation between surgeon performed AUS and PET/CT data with SLNB results to further determine the predictive role of AUS in pathologic staging of cN0 axilla after NAC.

Materials-Methods: A single institution, retrospective review of a prospectively maintained database was analyzed to identify ycN0 breast cancer patients with AUS and 18F-FDG PET/CT. All AUS studies were interpreted by a dedicated breast surgeon experienced in ultrasound, as “normal” according to the absence of specific characteristics shown to be commonly associated with metastatic involvement at diagnosis and at the date of operation. 18F-FDG PET/CT scans was termed as negative or positive due to the standardized uptake value (SUV). Patient, tumor and operative variables including age, body mass index (BMI), date of diagnosis and surgery, AUS, 18F-FDG PET/CT scans, SLNB results, and final pathology data were evaluated.

Results: Of the 69 patients with cN0 axilla after NAC, SNLB was found to be positive in 37 patients (53.6%). 2 (9.5%) out of 21 patients with a normal AUS and 3 (21.4%) out of 14 patients with negative PET/CT were ultimately found to be node-positive on pathologic assessment of SLNB. Intraoperative sonography accurately identified the SLN in 92.7% of cases. The sensitivity, specificity, positive and negative predictive values were 94.5%, 59.3%, 72.9% and 90.5% for surgeon-performed AUS and 91.8%, 34.4%, 61.8% and 78.6% for PET/CT scans, respectively. Overall accuracy was found to be %78.2 for AUS and 65.2.% for PET/CT. The presence of lymphovascular invasion (LVI), micrometastasis, primary tumor size, and body mass index were found to be significantly different between true and false negative AUS. None of the clinicopathological features of the primary tumor were significantly associated with FDG uptake in the axillary lesion. Micrometastatic disease, the size and number of metastatic nodes were significantly associated with FDG uptake leading to a difference between true and false negative PET/CT for axillary disease. No significant difference was noted with regard to patient age, tumor grade, histologic type, hormone receptor status, and time between AUS or PET/CT and axillary surgery.

Conclusion: Surgeon performed AUS is a beneficial tool with the potential of accurate prediction of axillary disease in up to 78% of patients after NAC. Nevertheless, the accuracy of AUS findings should be interrogated cautiously particularly for larger tumors with LVI or micrometastasis in overweight patients. Similarly, our data also imply that PET/CT had a limited value in the evaluation of axillary nodes and is not sufficient to predict axillary status particularly in case of micrometastasis after NAC.
Title: Re-evaluating the “10% rule” for sentinel lymph node biopsy with radioactive method in breast cancer; a single institutional retrospective study

Hiromi Miyamoto¹, Tomoyuki Aruga¹, Mai Onishi¹, Risa Goto¹, Naoko Iwamoto¹, Nami Idera¹, Kazumi Horiguchi¹ and Yayoi Honda¹. ¹Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, Bunkyo-ku, Tokyo, Japan.

Body: Background:
When multiple radioactive sentinel lymph nodes (SLNs) are present during sentinel lymph node biopsy (SLNB), excision of those SLNs with >10% radioactive count per minute (high-CPM) of the most radioactive node (“10% rule”) has been proposed. Although this “10% rule” may avoid excessive removal of SLNs, the risk of false negative and remnant positive SLNs in the patients who have SLNs with <10% CPM (low-CPM) remain unclear. The purpose of this analysis is to determine the clinical validity of this “10% rule” for early breast cancer patients.

Method:
We reviewed the records of successful SLNBs using the radioisotope (RI) method performed between January 2001 and December 2016 in our institution. The radioactive count from each excised SLN was measured. Non-radioactive lymph nodes were excluded from this analysis. All SLNs were pathologically assessed by 2mm serial section with hematoxylin and eosin staining.

Results:
In the 3,043 patients with successful SLNB, the median number of radioactive SLNs removed was 2 (mean, 1.8; range, 1-10) and 599 patients (19.7%) had SLNs with low-CPM. The total number of radioactive SLNs was 5,472, and 875 (16.0%) out of 5,472 SLNs were low-CPM. Sixty-one (7.0%) out of these 875 SLNs with low CPM in 56 patients (1.8%) had metastatic disease by pathological assessment. The number of metastatic SLN with low-CPM was one in 51 patients and two in 5 patients. Nineteen patients (0.6%) had no metastatic lesion in SLNs with high-CPM.

Discussion and Conclusions:
If SLNB was performed by RI method alone with “10% rule”, false negative rate increased by 0.6% and underestimation increased up to 1.8%. Furthermore, 19.7% of the patients have the benefit of avoiding excessive removal of SLNs. Considering the risk and benefit, “10% rule” is a high validity method to capture metastatic SLNs even in the setting that ALND will not be performed.
Publication Number: P3-01-10

Title: Can we predict the risk for non-sentinel node metastases? Results from the Swedish breast cancer registry on 23053 patients

Eva VikhePatil¹, Lars-Gunnar Arnesson¹ and Helena Fohlin². ¹University Hospital, Linkoping, Ostergotland, Sweden and ²Regional Cancer Centre, Linkoping, Ostergotland, Sweden.

Body: Introduction
Performing axillary clearance after node positive sentinel node biopsy (SNB) has been challenged. This register study aimed to predict the risk for non sentinel node metastases (non-SN) after axillary clearance in Swedish breast cancer patients. The Swedish Breast Cancer Registry has been in use since 2008 with >99% compliance. National guidelines recommended axillary clearance for macro-and micromets in SN but not for isolated tumor cells (ITC). Breast cancer screening is performed nationwide.

Methods
Registerdata for 33 314 patients, 2008 until May 2012, was evaluated. SN was performed in 23 053 patients corresponding to 69% of all patients, a stable figure since 2008. This cohort of 23053 patients is further investigated. The median age was 62 years. Breast conserving surgery (BCS) was performed in 61,5%. SN detection mode was radiocolloid and blue dye injection and often (41%) lymphoscintigraphy. Mean tumor size after BCS -16mm and in mastectomy (ME) -23 mm. BCS and ME show positive SN in 19% and 31% respectively (sign.<0.001). Number of excised nodes after axillary clearance was 13 (range 1-50). The dataset is not fully complete in all variables.

Results
Median harvested SNs was 2 (range 1-8). 5694 SN+ cases were found, distributed as14,9% macromets, 6,4% micromets and 2,0% ITC on the whole SN cohort. Screening detected cancers had metastatic SN in 18 % while clinical cases showed positivity in 28% (sign.). Altogether non-SN mets were found for 31% of SN positive patients. The risk of non-SN mets is: if 1 macromet in SN, 35% had further involved nodes. If 2 macromets in SNs, 49 % had non-SN mets and if ≥3 positive SNs the figure was 66% positive non-SN nodes (sign.<0.01). Evaluating 1299 SN micrometastatic cases gave these figures of freedom of non-SN mets: 1 micromet. node 86, 2 micromets. 77% was free and ≥3 micromets show 61,5% non-involved mets. These figures are also significant. Data on lymphovascular invasion (LVI) was available for 18754 cases and showed a significant higher risk for non-SN mets in LVI-positive tumors 43% against 27%. Tumours >20 mm and HER2 positive also show significant more non-SN mets.

Conclusion
The SN diagnostic technique works well in Sweden; 69% of patients had SN as the primary axillary procedure. SN+ was shown in 24,7%.The risk for non-SN mets is significant correlated to 2 or more involved SNs, positive LVI, tumour size >20 mm and HER2+. Is this the group for axillary surgery? We will try to get a Swedish risk score for non-SN+. A new Swedish national randomized study now investigates the need for axillary clearance in a subset of SN+ patients. Figures are collected from The Swedish Breast Cancer Registry-head K. Sandelin, Stockholm, Sweden.
Discoloration after injection of super paramagnetic iron oxide (SPIO) for sentinel node biopsy. A long term qualitative follow-up study

Madeleine Wärnberg¹, Andreas Karakatsanis¹, Shahin Abdsaleh² and Fredrik Wärnberg¹. ¹Uppsala Academic Hospital, Uppsala University, Uppsala, Sweden and ²Aleris, Uppsala University, Uppsala, Sweden.

Background

SPIO has a similar detection ratio as Technetium⁹⁹ and Patent Blue to identify sentinel nodes (SN). No allergic reactions have been observed and no nuclear medicine facilities are needed, making logistics easier. At the Academic Hospital in Uppsala, Sweden, SPIO has been used routinely for three years. After breast conserving surgery (BCS) many women developed a brownish discoloration at the injection site. The discoloration stays for a long time. To study the patient-experienced cosmetic discomfort and the natural history of the discoloration we followed our first 153 BCS women for more than two years. After modifying the technique of injection we registered the discoloration in the following 115 women.

Methods

All women injected with a retro-areolar injection of SPIO between April 2015 and October 2016 were included. The women were telephone interviewed every third month. The size of the discoloration was self-assessed and the cosmetic discomfort was classified by a scale from 0 (no discomfort) to 5 points (very discomforting). Photos were taken in selected cases after 1-2 years. Between November 2016 and April 2017, a deeper, para-tumoral injection was used and discoloration was noted 3 weeks after surgery.

Results

Ninety of 153 women (58.8%) developed a discoloration after a retro-areolar injection. The mean size was 26.1cm² (2-100cm²). The discoloration had vanished in 6.8% and 11.4% of the women after 1 and 2 years, respectively. The mean size of the discolorations was 18.2cm² (1-66cm²) and 13.6cm² (1-66cm²) after 1 and 2 years. The intensity of the color was continuously fading. The cosmetic discomfort was assessed as 2.2 points after 1 year and 1.0 after 2 years. Of 115 women with a deeper injection, 32.2% developed a discoloration with a mean size of 13.1cm² (1-36cm²), three weeks after surgery. The incidence and size of the discolorations were statistically significantly less and smaller after a deeper injection (p<0.001 and p=0.001, respectively). SNs were identified in 91% of women with a retro-areolar injection and in 93% of those with a deeper injection.

Conclusions

After an injection of SPIO, a discoloration might develop and the discoloration can stay for more than 2 years in many women. However, the color fades and the size diminish continuously and the women do not consider the discoloration a major cosmetic problem. A deeper injection reduces the incidence and the size of the discoloration with a similar detection ratio of SN. In our next study, we aim to reduce the volume of SPIO and thereby hopefully reduce the discoloration.

Patient-assessed cosmetic discomfort after 1 and 2 years

<table>
<thead>
<tr>
<th>Patient age</th>
<th>1 year</th>
<th>2 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;55 years</td>
<td>3.1</td>
<td>0.9</td>
</tr>
<tr>
<td>≥55 years</td>
<td>1.9</td>
<td>1.0</td>
</tr>
</tbody>
</table>

0=no discomfort, 5=very discomforting
Title: FNAC: A predictor of final number of involved nodes at axillary clearance

Ayesha Khan¹, Nadia Hussain¹ and Tracey Irvine¹. ¹Royal Surrey County Hospital, Guildford, United Kingdom.

Body: BACKGROUND:
A vital part of pre-operative breast cancer assessment is axillary staging. Fine needle aspiration cytology (FNAC) is performed on clinically or radiologically positive axillary nodes in breast cancer. Our study looks at the sensitivity of performing FNAC in these patients and whether positive FNAC of axillary nodes can predict the final number of diseased nodes on completion axillary clearance (ANC).

METHODS:
All primary breast cancer patients undergoing FNAC between Oct 2009 to Oct 2011 were identified from electronic computer records. Data was collected on FNAC positivity, whether sentinel lymph node biopsy (SLNB) was performed, total number of nodes harvested at ANC and also the total number of diseased nodes at ANC. Patients who underwent neoadjuvant chemotherapy after a positive FNAC were analysed separately.

RESULTS:
Of the 230 patients who underwent FNAC, 130 were positive (56.5%). Of those who had a negative FNAC, 32% had a positive SLNB. We compared the ANC results of those who were FNAC positive (Group 1) with those who were FNAC negative but SLNB positive (Group 2). There was no significant difference in the mean number of harvested nodes at ANC between the two groups (mean= 17, p=0.14 on t-test). There was a significant difference in the number of diseased nodes in group 1 (mean = 7 nodes) compared with group 2 (mean = 3 nodes). In group 1, 56% of patients had 4 ≥ diseased nodes compared with 8.5% of patients in group 2. Neoadjuvant chemotherapy was given to 28 patients with a positive FNA. In this cohort, the mean number of positive nodes at axillary clearance was 2 and 10 % of patients had 4 ≥ diseased nodes on ANC. There was no significant difference in mastectomy versus breast conserving surgery rates between FNA positive and negative patients (p= 0.28 on Chi Square). The sensitivity of performing an FNAC was 81% in this study.

Outcome of Patients undergoing Axillary Fine Needle Aspiration Cytology

<table>
<thead>
<tr>
<th></th>
<th>Group 1: FNAC positive</th>
<th>Group 2: FNAC negative, SLNB positive</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean No Harvested Nodes at ANC</td>
<td>18</td>
<td>16</td>
<td>0.14</td>
</tr>
<tr>
<td>Mean No of diseased nodes at ANC</td>
<td>7</td>
<td>3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>% of Patients with 4 or more diseased nodes at ANC</td>
<td>56</td>
<td>8.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>% of Patients who had Breast Conserving Surgery</td>
<td>54</td>
<td>53</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Key: FNAC: fine needle aspiration cytology, SLNB: Sentinel lymph node biopsy, ANC: Axillary clearance

CONCLUSION:
This study provides a predictor of the number of diseased axillary nodes in those with positive FNAC preoperatively. Over 50% of patients with a positive FNA have four or more diseased nodes compared with less than 10% in the FNAC negative but SLNB positive group. After neoadjuvant chemotherapy, FNA positive patients have a similar disease burden in the axilla as the FNAC negative but SLNB positive patients. This information can help to guide pre-operative discussions on the likely disease burden and need for adjuvant therapies such as chemotherapy and radiotherapy. Being able to predict the need for radiotherapy in particular can guide surgical decisions regarding type and timing of reconstructions to reduce the risk of surgical complications.
2017 San Antonio Breast Cancer Symposium

Publication Number: P3-01-14

Title: Nomogram predicting axillary lymph node metastases to skip intraoperative analysis of sentinel lymph nodes

Jung Hyun Park¹, Young Wook Ju¹, Kyoung Eun Kim¹, Jiyoung Rhu¹, Yumi Kim¹, Eunshin Lee¹, Han-Byoel Lee¹,², Hyeong-Gon Moon¹,², Dong-Young Noh¹,² and Wonshik Han¹,². ¹Seoul National University Hospital, Seoul, Korea and ²Cancer Research Institute, Seoul National University College of Medicine, Seoul, Korea.

Body: Background: According to the American College of Surgeons Oncology Group Z0011 trial, complete axillary lymph node dissection (ALND) did not affect survival of patients with clinical T1-T2 invasive breast cancer and one to two sentinel lymph nodes (SLNs) metastases treated with lumpectomy, adjuvant systemic therapy, and radiation therapy. A significant proportion of breast cancer patients may not require ALND, in whom intraoperative analysis of SLNs can be omitted reducing operation time and cost. The aim of this study was to develop a nomogram predicting three or more axillary lymph nodes (ALNs) metastases based on preoperative imaging and clinicopathological factors.

Methods: The training set consisted of 1030 patients with clinical T1-T2 invasive breast cancer and clinically negative ALN who received surgery at Seoul National University Hospital (SNUH) between January 2010 and December 2013. Preoperative imaging techniques including ultrasonography (US), computed tomography (CT), positron emission tomography (PET), and clinicopathological features associated with three or more ALN metastases were evaluated by logistic regression analysis. A nomogram predicting three or more ALNs was developed with statistically significant factors. The validation set consisted of 781 independent patients who received surgery at SNUH between January 2014 and December 2015.

Results: Of the 1030 patients, 89 (8.6%) had three or more ALN metastases. Multivariate analysis showed that three or more ALN metastases was independently associated with tumor size (cm) by US (p<0.001), suspicious ALNs findings in US (p<0.001), chest CT (p<0.001), and PET/CT (≥ 1.4 SUV, p<0.001). Established nomogram evaluating the probability of three or more ALNs metastases includes the above four associated factors. The areas under the receiver operating characteristic (ROC) curve of the nomogram were 0.866 (95% confidence interval [CI] 0.826-0.905) for the training set and 0.867 (95% CI: 0.801-0.932) for the validation set. With cutoff point of 142, false negative ratio is 3.6%, and 8.6% of patients were candidates for intraoperative SLN analysis.

Conclusion: Patients with a strong possibility of three or more ALNs metastases can be identified using preoperative imaging methods including US, CT, and PET. The nomogram measuring this prospect may be valuable in skipping intraoperative analysis of SLNs with advantage of reduced operation time and cost.
2017 San Antonio Breast Cancer Symposium

Publication Number: P3-01-15

Title: Sentinel lymph node biopsy by indocyanine green fluorescence detection in breast cancer cT1-4N0M0 patients. Frequency of false-negative results is low


Body: Breast cancer cT1-4N0M0 patients are usually need in a sentinel lymph node biopsy (SLNB). SLNB by indocyanine green (ICG) fluorescence detection is a modern technique possessing high level of lymph node detection rate, but frequency of false-negative results was not evaluated adequately.

Objective was to determine main diagnostic characteristics of the ICG fluorescence-guided SLNB in BC cT1-4N0M0 patients: node detection rate and frequency of false-negative results.

Methods: 99 patients with 100 cases of breast cancer cT1-4N0M0 (T1 – 43, T2 – 55, T3 – 1, T4 – 1) were operated, axillary part of operation consisted from the ICG fluorescence-guided SLNB and axillary lymphadenectomy of levels I–II or I–II–III. 12 patients had neoadjuvant chemotherapy and 8 – neoadjuvant hormonotherapy. Photodynamic Eye C9830 (Hamamatsu Photonics K.K) was using for emit near infrared radiation and fluorescent image registration. 2 ml 0.25% solution of ICG were injected inra- and sub-cutaneous periareolary or in a tumor projection, 2-5 minutes later it can be see fluorescent way to axillary. The procedure starting from injection of the ICG to the identification of a signal lymph node takes 15–30 minutes.

Results. Transcutaneous fluorescent lymphatic duct was visible in all 100 procedures, but not lymphatic node. Last can be found usually in a wound after Fascia superficialis and Fascia axillaris dissection. A signal lymph node was detected in 98 cases (98 %). Mean amount of sentinel lymphatic nodes = 1.9±0.1 (1-7 nodes). Mean amount of lymphatic nodes took with axillary dissection = 10.5±0.5 (5-26 nodes). In 28 (28.6%) cases of 98 were found to have metastases in signal lymph nodes. Other than signal lymph nodes had metastatic lesion only in 35.7% in signal lymph nodes N+ cases. False negative result occurred in 3 (3.1%) cases of 98, including 2 with metastases in signal lymph node finding by plan histological investigation, but not at froze section. No cases of allergic reactions or other adverse effects were diagnosed with standard subcutaneous use of indocyanine.

Conclusion. Fluorescence technique of the detection of signal lymph nodes has its own methodological issues: in most cases a signal lymph node is not visualized through the skin, it should be visualized in surgical wound using the course of lymphatic duct as guidance. Application of ICG fluorescence-guided SLNB in cN0 breast cancer patients allows high signal lymph node detection rate and low false negative rate, - 3%.

Acknowledgments. The authors thank Professor Gordon C. Wishart for the Sentinel Lymph Node Detection by ICG Fluorescence Technique teaching.
Title: How hot is enough for accurate sentinel lymph node axillary staging in breast cancer?

Moo Hyun Lee¹, Sun Hee Kang¹ and Jihyoung Cho¹. ¹Keimyung University School of Medicine, Daegu, Korea.

Body: Purpose:
Sentinel lymph node (SLN) biopsy is a well-established procedure for staging of the axilla in early-stage breast cancer and has replaced axillary lymph node dissection as the standard of care in patients with clinically lymph node–negative axilla. No consensus exists about the number of sentinel lymph nodes (SLNs) that should be removed based on radioactivity counts in breast cancer, although the “10% rule” is often used. In order to determine the frequency with which the hottest SLN 'fails' to be pathologically positive, and to determine which criteria best define the radioactive lymph node to be removed, we reviewed and analyzed our cases in which more than one SLN was detected and where there was also at least one pathologically positive node.

Methods:
We retrospectively studied 1062 breast cancer patients who underwent lymphoscintigraphy by injection of radioactive colloid and SLN biopsy between 2006 and 2015, with intraoperative determination of radioactive counts of nodes by a gamma probe.

Results:
A total of 247 patients (23.3%) had more than 1 SLN removed (mean 2.29); 53 patients (21.5%) had nodal metastases. Of the node-positive patients, the hottest SLN was positive in 90.6% (48 of 53). The lowest radioactive count of a positive SLN was 32% of that of the hottest node.

Conclusions:
In our study, most positive SLNs had the highest radioactivity and the hottest lymph node was not the pathologically positive node only in 9.4 %. Our institutional experience indicates that to obtain an acceptable false-negative rate, nodes should be removed until the 10% rule is met.
Title: Evaluation of “Systematic sonographic axillary staging” on clinically node positive breast cancer patients becoming clinically node negative after neoadjuvant chemotherapy

Tolga Ozmen¹, Mesa Lazaro¹, Alicia Vinyard¹ and Eli Avisar¹. ¹University of Miami, Miller School of Medicine, Miami, FL.

Body: Purpose
Accumulating evidence supports usage of sentinel lymph node biopsy (SLNB) for clinically node positive (cN+) breast cancer (BC) patients, who become clinically node negative (ycN-) after neoadjuvant chemotherapy (NAC). How to reassess the axilla after NAC is a challenging issue. In our institution, Systematic Sonographic Axillary Staging (SSAS) is currently being tested in this group of patients. The aim of this study is to analyze the early results of this approach.

Method
During initial presentation, systematic sonographic axillary staging is done preferentially by the surgeon. Needle biopsy is then performed of suspicious node(s). At completion of NAC, the axilla is again systematically staged sonographically, taking into account the pre-treatment disease locations. Any residual morphologically suspicious node undergoes a repeat needle biopsy. According to the evidence of residual disease, the patient is either scheduled for axillary dissection (AD) or for SLNB. We analyzed early results of SSAS on patients, who converted ycN (-) after NAC. Strictly adherence to SSAS protocol was the only inclusion criteria.

Results
A total of 25 patients were included. On repeat axillary USG after NAC, 11 patients had normal appearing and 14 patients had suspicious appearing lymph nodes (LNs). 14 patients underwent repeat biopsy; 9 patients had benign and 5 patients had malign pathology results. 20 patients underwent SLNB. Among 20 patients, 12 patients had negative SLNB and 8 patients had positive SLNB (4 patients had benign appearing LNs on repeat USG and 4 patients had benign biopsy results). 13 patients underwent AD (8 patients after positive SLNB and 5 patients directly after positive US-guided biopsy result). Among 8 patients with positive SLNB, 4 patients had macrometastatic disease (>2mm) on SLNB. In 3 of these 8 patients, no additional disease was found on AD, while 1 patient had only isolated tumor cell and 1 patient had only micrometastasis (Table 1).

Table 1. Size of the axillary involvement among patients with positive SLNB

<table>
<thead>
<tr>
<th>Patient #</th>
<th>LN* (+) / Total LNs removed</th>
<th>Size of involvement (mm)</th>
<th>LN* (+) / Total LNs removed</th>
<th>Size of involvement (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1/6</td>
<td>1</td>
<td>1/5</td>
<td>0.1</td>
</tr>
<tr>
<td>2</td>
<td>2/4</td>
<td>2.5</td>
<td>0/5</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>1/3</td>
<td>6</td>
<td>3/19</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>1/2</td>
<td>1</td>
<td>0/3</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>1/1</td>
<td>1.5</td>
<td>0/21</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>2/2</td>
<td>1.5</td>
<td>3/3</td>
<td>2.5</td>
</tr>
<tr>
<td>7</td>
<td>3/3</td>
<td>5</td>
<td>7/10</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>4/4</td>
<td>4</td>
<td>7/13</td>
<td>3</td>
</tr>
</tbody>
</table>

* LN = lymph node

Conclusion
Systematic sonographic axillary staging is a reliable approach to cN (+) patients, who become ycN0 after NAC. This observation should be tested in a larger study.
Title: Determining the need for axillary lymph node dissection based on pre-neoadjuvant chemotherapy sentinel lymph node biopsy results

Vladimir F Semiglazov¹, Petr V Krivorotko¹, Elena K Zhiltsova¹, Garik A Dashyan¹, Konstantin U Zernov¹, Alexandr A Bessonov¹, Eketerina S Trufanova¹, Tengiz T Tabagua¹, Olga A Ivanova¹, Sergey V Kanaev¹, Sergey N Novikov¹, Pavel I Krzivickiyi¹, Zanna V Brayanceva¹, Alexander V Komayachov¹, Kirill S Nikolaev¹ and Larisa P Gigolaeva¹. ¹N.Petrov Research Institute of Oncology, St.Petersburg, Russian Federation.

Body: An open-label single institution study of sentinel lymph node biopsy prior to neoadjuvant chemotherapy is conducted in the N.N. Petrov research institute of oncology. So far 30 patients with locally advanced breast carcinoma (T2-4N0) were included. Mammography, breast US and SPECT are performed prior to hospitalization so as core biopsy with subsequent IHC staining to determine the levels of ER, PR, Ki67 and HER2. Sentinel lymph node biopsy with the use of radiocompound is performed prior to the first cycle of neoadjuvant chemotherapy. Patients receive 4 to 6 cycles with response evaluation after cycles 2, 4 and 6 (same evaluations as at the baseline). Axillary lymph node dissection is later performed along with the definitive surgery of the primary. Interim analysis showed: 3 of 30 patients with cN0 were node positive after sentinel lymph node biopsy, 27 were node-negative (sn)pN0. All patients with (sn)pN0 completed neoadjuvant chemotherapy with clinical response. No cases of upfront progression were detected. All patients (sn)pN0 underwent axillary dissection upon completion of the neoadjuvant therapy. No cases of nodal involvement were detected (ypN0). All of the patients considered node-positive after sentinel biopsy also underwent lymph node dissection after completion of the neoadjuvant therapy. 2 of them were pN1 and one was pN2.

Conclusion: sentinel lymph node biopsy in cN0 patients prior to neoadjuvant chemotherapy allows to determine a category of patients ((sn)pN0) in whom axillary dissection can be avoided, provided they remain clinically node-negative at the time of definitive surgery.
Title: Relationship of axillary total tumor load (TTL) by OSNA (one step nucleic acid amplification) in early breast cancer and clinical outcomes using strict Z0011 study criteria for axilla management

Raquel Tur1, Carlos De Grado2, María Rocío Martin1, Javier De Castro3, Elena Filipovich4, Beatriz Segovia1, Jaime Ceballos4, Juan Parrá5, Rafael Revestido1 and José E Alés-Martínez4. 1Complejo Asistencial de Ávila, Ávila, Spain; 2Complejo Asistencial de Ávila, Ávila, Spain; 3Complejo Asistencial de Ávila, Ávila, Spain; 4Complejo Asistencial de Ávila, Ávila, Spain and 5Biomedical Investigation Center (CIBER-BBN. ISCIII), Ávila, Spain.

Body: Background: The study of sentinel lymph node (SNL) assessed by OSNA provides a new variable, Total Tumoral Load (TTL). This variable is defined as the amount of CK19 mRNA copies number in all positives SLN. TTL has been showed to predict the axillary node status and has been analysed to determine its usefulness in the axillary surgical management. Based on TTL values different cut-off points have been proposed (last 25,000 copies) to establish a new tool to practice axillary lymph node dissection (ALND). We present the follow-up data of at least 5 years of breast cancer patients who underwent ALND according, strictly, to Z0011 trial criteria. We hypothesized that there will be no correlation between TTL and locoregional relapse if Z0011 are followed.

Methods: Clinicopathological and follow up data were obtained from patients with invasive breast cancer and SNL assessed by OSNA between 2011 and 2012 at Complejo Asistencial de Ávila, Spain. ALND was decided based on Z0011 study criteria independently of TTL. All patients have been followed for a minimum of 5 years.

Results: A total of 106 patients underwent SN assessed by OSNA, age range 27-85 years (mean 58.96). Of them 90% were ductal, 7.5% lobular and 2% others. By immunophenotype: Luminal A 55%, Luminal B 28%, Triple Negative 9.4%, Her2 positive 3.7% and Luminal B-Her2 positive 2.8%. TTL was equal to zero in 58 cases and greater than zero in 48 cases with a range of 280-2.700.000 copies. Only 5 cases met ALND criteria (average TTL 68.164). Average TTL in cases without ALND was 111.000. For the time being, none of them has had locoregional relapse (median follow up 65 months). 3 patients have died one metastatic disease (Negative SN), one uterine cervix cancer and one neutropenic fever.

Baseline and outcomes data

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (median, range)</td>
<td>59</td>
<td>27-85</td>
</tr>
<tr>
<td>Tumour Type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ductal</td>
<td>96</td>
<td>90,5</td>
</tr>
<tr>
<td>Lobular</td>
<td>8</td>
<td>7,5</td>
</tr>
<tr>
<td>Others</td>
<td>2</td>
<td>1,8</td>
</tr>
<tr>
<td>Inmunophenotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luminal A</td>
<td>59</td>
<td>55,6</td>
</tr>
<tr>
<td>Luminal B</td>
<td>30</td>
<td>28,3</td>
</tr>
<tr>
<td>Luminal B-Her2</td>
<td>3</td>
<td>2,8</td>
</tr>
<tr>
<td>Her2</td>
<td>4</td>
<td>3,7</td>
</tr>
<tr>
<td>Triple Negative</td>
<td>10</td>
<td>9,4</td>
</tr>
<tr>
<td>Total Tumoral Load (TTL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>=0</td>
<td>58</td>
<td>54,7</td>
</tr>
<tr>
<td>&gt;0</td>
<td>48</td>
<td>45,2</td>
</tr>
<tr>
<td>Axillary Lymph Node Dissection (ALND)</td>
<td>25</td>
<td>4,7</td>
</tr>
<tr>
<td>TTL &gt;25.000</td>
<td>23</td>
<td>21,7</td>
</tr>
<tr>
<td>Locoregional relapse</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Overall Survival</td>
<td>95,2</td>
<td></td>
</tr>
</tbody>
</table>
Conclusions:
- Using Z0011 criteria and OSNA no locoregional recurrence has been observed so far.
- TTL did not predict risk of recurrence
- If we had based axillary management only on TTL values (i.e. higher than 25,000 copies) we would have unnecessarily increased the number of lymphadenectomies in a 22%.
This is an ongoing study that designed to increased the sample size and obtain longer follow-up data.
**Title:** Impact of prognoses on the discrepancies in histological grade of breast cancer between core needle biopsy and surgical excision specimen

Rikiya Nakamura¹, Naohito Yamamoto¹, Toshiko Miyaki¹, Ryoutarou Teranaka¹ and Makiko Itami². ¹Chiba Cancer Center, Chiba, Japan and ²Chiba Cancer Center, Japan.

**Body:** Purpose: The high reliability and utility of core needle biopsy (CNB) have been previously described. Histological grade in CNB is one of the main determinants of the need for neoadjuvant systemic therapy. Our aim in this study was to clarify the host and histopathological factors influencing the discrepancies in histological grade (HG) between CNB and surgically excision specimen (SES).

Methods: A total of 1342 operable invasive breast carcinoma biopsies were assessed and compared with surgical specimens in our hospital. Patients who required neoadjuvant chemotherapy were excluded. Histological grade (tubule formation, nuclear pleomorphism and mitotic index) was assessed between paired CNB and SET samples.

ER and PgR status were determined using immunohistochemistry(IHC). HER2 status was determined using IHC and scored from 0 to 3+. Fluorescence in-situ hybridization analysis was carried out in HER2 2+ cases. The cut off point for ER and PgR positivity was set at 1%.

Results: The clinicopathological characteristics of tumors showed in

Clinico-pathological characteristics of 1342 patients and tumors by discordance group between CNB and SES for histological grade

<table>
<thead>
<tr>
<th></th>
<th>LL group</th>
<th>HH group</th>
<th>HL group</th>
<th>LLH group</th>
<th>p valu</th>
</tr>
</thead>
<tbody>
<tr>
<td>pT</td>
<td>T1a</td>
<td>31 (94%)</td>
<td>0 (0%)</td>
<td>2 (6%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td></td>
<td>T1b</td>
<td>162 (92%)</td>
<td>4 (2%)</td>
<td>1 (1%)</td>
<td>10 (6%)</td>
</tr>
<tr>
<td></td>
<td>T1c</td>
<td>421 (79%)</td>
<td>43 (8%)</td>
<td>14 (3%)</td>
<td>52 (10%)</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>335 (68%)</td>
<td>63 (13%)</td>
<td>10 (2%)</td>
<td>82 (17%)</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>52 (68%)</td>
<td>8 (10%)</td>
<td>2 (3%)</td>
<td>15 (19%)</td>
</tr>
<tr>
<td></td>
<td>T4</td>
<td>25 (71%)</td>
<td>4 (11%)</td>
<td>0 (0%)</td>
<td>6 (17%)</td>
</tr>
<tr>
<td>pN</td>
<td>negative</td>
<td>668 (78%)</td>
<td>73 (9%)</td>
<td>13 (2%)</td>
<td>101 (12%)</td>
</tr>
<tr>
<td></td>
<td>positive</td>
<td>358 (74%)</td>
<td>49 (10%)</td>
<td>16 (3%)</td>
<td>64 (13%)</td>
</tr>
<tr>
<td>ly</td>
<td>negative</td>
<td>600 (79%)</td>
<td>64 (8%)</td>
<td>14 (2%)</td>
<td>85 (11%)</td>
</tr>
<tr>
<td></td>
<td>positive</td>
<td>426 (74%)</td>
<td>58 (10%)</td>
<td>15 (3%)</td>
<td>80 (14%)</td>
</tr>
<tr>
<td>v</td>
<td>negative</td>
<td>799 (80%)</td>
<td>81 (5%)</td>
<td>23 (2%)</td>
<td>98 (10%)</td>
</tr>
<tr>
<td></td>
<td>positive</td>
<td>227 (67%)</td>
<td>41 (12%)</td>
<td>6 (2%)</td>
<td>67 (20%)</td>
</tr>
<tr>
<td>ER/PgR</td>
<td>negative</td>
<td>82 (33%)</td>
<td>83 (33%)</td>
<td>13 (5%)</td>
<td>72 (29%)</td>
</tr>
<tr>
<td></td>
<td>positive</td>
<td>944 (86%)</td>
<td>39 (4%)</td>
<td>16 (1%)</td>
<td>93 (9%)</td>
</tr>
<tr>
<td>HER2</td>
<td>negative</td>
<td>932 (80%)</td>
<td>86 (7%)</td>
<td>22 (2%)</td>
<td>121 (10%)</td>
</tr>
<tr>
<td></td>
<td>positive</td>
<td>94 (52%)</td>
<td>36 (20%)</td>
<td>7 (4%)</td>
<td>44 (24%)</td>
</tr>
</tbody>
</table>

CNB; core needle biopsy, SES; surgical excision specimen, HH group (high grade in CNB/high grade in SES), LL group (low grade in CNB/low grade in SES), HL group (high grade in CNB/low grade in SES) and LH group (low grade in CNB/high grade in SES).

The concordance rates of HG of luminal type, HER2 type luminal HER2 type and Triple negative type in CNB and SES were 91%, 64%, 73% and 66%, respectively (p>0.001). Factors of discrepancy were T size, vessel invasion and ER/HER2 status for HG. The discrepancy factors were assessed with univariate and multivariate analysis. The underestimate and overestimate rates of HG in CNB compared to SES were 7.5% and 1.3% in ER(+)HER2(-) type, 32%, and 3.1% in HER2 type, 22% and 4.3% in
ER(+) HER2(+) type and 29% and 5.5% in Triple negative type (TN), respectively. The concordance rates of tubule formation, nuclear pleomorphism and mitotic index in CNB and SES were 81%, 97% and 93% in luminal type, 85%, 66% and 33% in HER2 type, 77%, 87% and 83% in luminal HER2 type and 82%, 67% and 73% in TN type respectively.

Conclusions: Using the largest known dataset to date of paired samples from a single institution, we evaluated the accuracy of CNB and the discrepancy factors between CNB and SES in breast cancer patients.

We conclude that CNB for histological grade assessment in patients with HER2 positive or TN breast cancer before neoadjuvant treatment should be used with caution.
2017 San Antonio Breast Cancer Symposium

Title: Concordance between immunohistochemical and gene-expression based subtyping of early breast cancer using core needle biopsies and surgical specimens - experiences from SCAN-B

Gilberto Morgan¹, Christer Larsson³, Balázs Tahin⁵, Johan Vallon-Christersson²,³, Jari Häkkinen²,³, Anna Ehinger²,³,⁴, Martin Malmberg¹, Cecilia Hegardt²,³, Åke Borg²,³, Lisa Rydén²,⁷, Lao H Saal²,³, Ingrid Hedenfalk²,³ and Niklas Loman¹,²,³. ¹Skåne University Hospital, Lund, Sweden; ²Lund University, Lund, Sweden; ³Lund University Cancer Centre, Medicon Village, Lund, Sweden; ⁴Skåne University Hospital, Lund, Sweden; ⁵Skåne University Hospital, Malmö, Sweden; ⁶Lund University, Lund, Sweden and ⁷Skåne University Hospital, Lund, Sweden.

Body: Introduction: Preoperative chemotherapy in early breast cancer increases the rate of breast preservation and provides prognostic information. Treatment decisions in these cases rely on biomarker assessments and subtyping from tissue acquired through core needle biopsies. Tumor heterogeneity and representativity are pitfalls when limited tissue is available. Biomarker expression may change considerably as a result of preoperative chemotherapy, and in a subset of cases a complete pathological response at time of surgery may even preclude any further assessment. Therefore, the reliability and reproducibility of biomarkers in base-line core biopsies are of utmost importance for patients treated with preoperative chemotherapy.

Material and Methods: In an ongoing population-based study of early breast cancer, the SCAN-B (NCT02306096), patients were identified for whom an ultra-sound guided core needle biopsy was analyzed for biomarkers during primary clinical work-up and the patient was offered primary surgery as initial treatment. Clinical biomarker profiles including immunohistochemical (IHC) determinations of ER, PgR, HER2 and Ki67 were translated to subtypes according to modified St Gallen criteria (2013) and compared with paired samples from surgical specimens. In addition, tumor specimens for biomolecule extraction and RNA sequencing were collected fresh in RNAlater.

Results: IHC data was available from 51 paired samples. The subtype distribution in core needle biopsies was DCIS in 1 case (2 %), LCIS in 1 case (2 %) Luminal A-like in 16 cases (31 %), Luminal B-like (HER2 negative) in 26 cases (51 %), Luminal B-HER2-like (HER2 positive) in 4 cases (8 %), HER2-positive (non-luminal) in 1 case (2 %) and triple negative (ductal) breast cancer in 2 cases (4 %). The subtype distribution in surgical specimens was DCIS in 0 case (0 %), LCIS in 1 case (2 %) Luminal A-like in 18 cases (35 %), Luminal B-like (HER2negative) in 23 cases (45 %), Luminal B--like (HER2 positive) in 6 cases (12 %), HER2-positive (non-luminal) in 1 case (2 %) and triple negative (ductal) breast cancer in 2 cases (4 %). Notably, 5/16 cases classified as Luminal A-like in the core needle biopsy were reclassified as Luminal B-like (HER2-negative) in the surgical specimen, whereas 9/26 cases classified as Luminal B-like (HER2-negative) in the core needle biopsy were reclassified as either Luminal A-like (7 cases) or Luminal B-like (HER2 positive) (2 cases) in the surgical specimen. In all instances, except one, transition between Luminal A-like and Luminal B-like was due to recorded Ki67 expression. One case that was classified as a DCIS in the core needle was reclassified as Luminal B-like (HER2 negative) at time of surgery.

Discussion: In this limited material, discordance between evaluations regarding Luminal A-like and Luminal B-like was considerable. Especially the misclassification of primary HER2-positive breast cancer needs further evaluation. These findings may be caused by tumor heterogeneity, and highlight the risk of both over- and under-treatment upon biomarker assessment from core needle biopsies. Data from gene expression based subtype classifications will be presented during the meeting.
2017 San Antonio Breast Cancer Symposium

Publication Number: P3-02-03

Title: Detection of mutations in single tumor cells collected by fine needle aspiration in a mouse xenograft breast cancer model using MALDI-TOF

Jackie L Stilwell¹, Amy Hobeida², Ryan T Birse³, Nolan Ericson¹, Arturo B Ramirez¹, Sara Hummel³, Darryl Irwin³, Eric P Kaldjian¹ and H Kim Lyerly². ¹RareCyte, Inc, Seattle, WA; ²Duke University Medical Center, Durham, NC and ³Agena Bioscience, Inc, San Diego, CA.

Body: Background: Breast fine needle aspiration (FNA) is less invasive than a core needle biopsy and reduces the risk of infection or injury to the patient. However, less tissue is available for analysis than with biopsy. The ability to detect and analyze single atypical cells for molecular abnormalities would allow the consideration of more widely adopting FNA for diagnosis. Here we demonstrate the feasibility of this approach in a mouse xenograft model of breast cancer.

Methods: Human-into-mouse MBA-MD-231 breast cancer xenograft tumors were aspirated using a technique that approximates the clinical procedure in patients. Cells from the FNA were prepared by two methods: 1) mixing the aspirate with transfer fluid and spreading onto a Superfrost® Plus slide using RareCyte’s AccuCyte® process, and 2) spraying the aspirate directly onto a Superfrost Plus slide, then drying and fixing in ethanol. A single tumor was also disaggregated into suspension, filtered, mixed with transfer fluid and spread on to slides as in method 1 above as a control. Slides were fixed in formalin, stained on an automated immunostainer and imaged using the CyteFinder® digital fluorescence scanning microscope. Tumor cells were identified by positive nuclear, EpCAM, and cytokeratin staining, and negative CD45 staining. Tumor cells were picked from the slides and put into PCR tubes using the CytePicker® module. DNA from individual cells was amplified using the PicoPLEX® (Rubicon) whole genome amplification (WGA) kit. Quality control (QC) of the WGA reactions was performed by PCR of amplicons on eight different chromosomes. Specific gene regions surrounding 5 mutations present in MBA-MD-231 cells were amplified from the WGA products and scored for the mutations using a single PCR reaction iPLEX® Pro panel using the MassARRAY® platform (Agena Bioscience). A lung tumor panel was also run as a negative control.

Results: FNA tumor cells stained with epithelial markers similarly to cells from the disaggregated tumor control and were easily identified. Slides prepared by method 1 above spread into a uniform monolayer making it easier to pick individual cells. Cells from method 2 tended to clump making it more difficult to pick individual cells. Cells were thus picked only from method 1 slides. QC measurements of WGA products from individual cells demonstrated broad genome coverage of amplification; 10 of 14 cells exhibited 7 or more QC products out of 8. Point mutations in four genes (BRAF, KRAS, NF2, and TP53) and a deletion in one gene (CDKN2A) were measured in these cells by iPLEX® Pro chemistry on the MassARRAY® system and found in all cells picked, with all mutations identified in most cells. These mutations and the deletion were not detected in control WBCs.

Conclusions: Individual breast cancer cells were identified in FNA samples from xenograft tumors and molecularly characterized, verifying that the cells identified by positive staining were tumor cells. These results demonstrate the feasibility of detecting and verifying tumor cells in FNA samples in breast and other cancers.
Clinical utility of finding pathogenic mutations beyond BRCA1/2 in breast cancer patients

Edward D Esplin¹, Scott Michalski¹, Shan Yang¹, Heather Hampel², Joanne Jeter², Kevin Sweet², Robert Pilarski², Rachel Pearlman², Kate Shane², Pamela Brock², Judith Westman², Anu Chittenden³, Jill Stopfer³, Katherine Schneider³, Rosalba Sacca³, Samantha Stickevers³, Lindsay Kipnis³, Diane Koeller³, Shraddha Gaonkar³, Jilliane Sotelo³, Erica Vaccari³, Sarah Cochrane³, Marjan Champine⁴, Whitney Espinel⁴, Stephen E Lincoln¹ and Robert L Nussbaum¹. ¹Invitae, San Francisco, CA; ²The Ohio State University Comprehensive Cancer Center, Columbus, OH; ³Dana Farber Cancer Institute, Boston, MA and ⁴Huntsman Cancer Institute, Salt Lake City, UT.

Body: Background

The clinical utility of germline genetic testing for BRCA1 and BRCA2 has long been established. However, management recommendations for pathogenic variants in other genes, typically included in multigene panels, have only recently been included in consensus guidelines for HBOC. The clinician actions implemented for findings in these genes, and patient follow-up, are not yet well studied. We report interim results from a multi-site study of clinical actions undertaken in patients presenting with HBOC and carrying a pathogenic germline mutation in cancer risk genes other than BRCA1/2.

Methods

We retrospectively examined a cohort of patients with a personal history of HBOC who had been referred for hereditary cancer multigene testing from three major academic medical centers. For patients with pathogenic findings in a non-BRCA1/2 cancer risk gene, ordering clinicians completed a short case report form describing the clinical actions taken in response to the genetic test result, and patient follow-up. Some patients were lost to follow-up and answers of “unknown” were permitted. Genes with positive findings included CHEK2, PALB2, ATM, MUTYH, RAD51C, TP53, MSH6, RAD50, APC, BARD1, BRIP1, MSH2, NF1, NBN, PMS2, and PTEN. Case report forms were available for 77 patients as of our cut off date, and these data were de-identified and summarized for this interim report. Additional cases continue to accrue in this ongoing study.

Results

In 57% (44/77) of cases, clinicians reported that counseling and/or clinical management recommendations were changed in response to the genetic test findings. Management changes included modification of imaging surveillance (38%), considered or recommended surgical prophylaxis (12%), modified surgical plan for an existing malignancy (5%), and for one patient each: inclusion in a research trial for PARP inhibitors, modification of colonoscopy schedule, and screening for cancers other than existing malignancy. Clinicians indicated that genetic test results changed management in 48% of patients, did not change management in 29%, and had unknown impact for 23%.

Clinicians also reported that counseling and/or management for the patients' family members was changed in 67% (52/77) of cases, including family variant testing. 27% (21/77) of the patient families had cascade genetic testing, and one or more new carriers were identified in 47% (10) of the tested families. In 58% of cases, the impact of management recommendations on family members was unknown as of the case report date.

Conclusions

Pathogenic variants in non-BRCA genes are present in about 3-11% of patients with a history of HBOC. This study suggests that genetic test results in cancer genes beyond BRCA1/2 changed clinical management for a majority of patients and their family members, led to identification of new carriers, and directly impacted treatment decisions. In almost half of these patients, genetic test results impacted their health outcome, including those reported to be disease free after undergoing intervention or prophylactic surgery informed by their genetic variant. More research is needed to improve the implementation of genetic testing based management recommendations for patients and their family members.
Title: ER dependent breast cancer phenotype in BRCA 1/2 carriers

Tamar Y Peretz¹, Aviad Zick¹, Kadouri Luna¹, Albert Grinshpun¹, Amir Sonneblick¹, Beatrice Iziely¹, Tamar G Hamburger¹, Sherri Cohen¹, Avital M Granit¹, Michal Dvir¹ and Shai Rosenberg¹. ¹Hadassah-Hebrew University Medical Center, Jerusalem, Israel.

Body: Background: Hereditary breast cancer (HBC) comprises more than 10% of all breast cancers (BC). Mutations in the BRCA1/2 genes are found in approximately half of HBC patients. The majority of BRCA1 associated tumors are ER, PR and HER2 negative with basal-like expression pattern. BRCA2 associated tumors are mostly ER positive (ER+). In the present study we aim to further explore clinical and molecular characteristics of BRCA associated BC in 3 different cohorts.

Methods: Three different BC databases (DB) were evaluated: (i) Hadassah oncogenetic BC DB (n=4429); (ii) Nick-Zainal et al. BC DB (n=560), and (iii) METABRIC BC DB (n= 1980). We tested for differences in age at diagnosis between BRCA positive (BRCA+) and BRCA negative (BRCA-) patients with either ER+ or ER negative (ER-) tumors. Point mutation analysis was performed in cohorts ii & iii and mRNA differential expression (DEA) and pathway analysis were performed in cohort iii, using Ingenuity Pathway Analysis (IPA).

Results: Age (years) at diagnosis for cohorts i, ii,&iii respectively, for ER+ PT: BRCA1-44, NA, 60; for BRCA2-49, 48, 64; for BN – 53, 56, 63. For ER-: BRCA1-42, 42, 47; for BRCA2-48, 52, 49; for BN-49, 54, 56. For cohorts ii&iii, higher frequencies of TP53 and PIK3Ca mutations were found among BRCA+&BRCA-, respectively. DEA was performed between BRCA++BRCA- in ER-tumors: the major activated pathways involved cancer related processes and were highly significant (up to p=1e-7.5, FDR=1e-4.5). Surprisingly - the most significant pathway was Estrogen Mediated S-phase Entry and the most activated upstream regulator was ERBB2. Similar evaluation in ER+ showed mostly differences in immune related pathways (differences not statistically significant).

Conclusions: Younger age at presentation was observed in BRCA1 vs. BRCA2 patients. No age differences were observed between ER+&ER- PT in cohort i&ii, in cohort iii ER- BRCA+ Patients were younger than ER+ BRCA+ (similar age as ER+ BRCA-). BRCA+ show different mutational profile than BRCA-. ER+ BRCA+ and BRCA- show similar genomic characteristics. By contrast, for ER- BRCA+ differs markedly from BRCA-. This might imply that BRCA+ associated tumors consist of two genomically distinct subtypes: (i) ER-, and (ii) ER+. The results may shed light on possible somatic factors which affect the development of BC BRCA+ and carry preventive and therapeutic implications.
Title: Analysis of hereditary cancer syndromes by use of a panel of genes: More answers than questions

Nikolaos Tsoulos¹, Angela Apessos¹, Konstantinos Agiannitopoulos¹, Georgia Pepe³, Georgios Tsiaousis¹, Stavroula Kambouri¹, Dan Tudor Eniu², Andrei Ungureanu³, Eugeni Banu³, Larisa Ciule³, Alexandru Blidaru³, Angelica Chiorean³, Dana Lucia Stanculeanu³, Delia Mateescu⁷ and Georgios Nasioulas¹. ¹GeneKor M.S.A, Athens, Attiki, Greece; ²Institutul Oncologic Prof. Dr. I. Chiricuta, CLUJ, Romania; ³Amethyst Radiotherapy, Cluj-Napoca, Romania; ⁴Spitalul Sfantul Constantin, Brasov, Romania; ⁵Spitalul Clinic Judetean de Urgenta, Cluj-Napoca, Romania; ⁶Institutul Oncologic Bucuresti, Bucuresti, Romania and ⁷Regina Maria, Bucuresti, Romania.

Body: INTRODUCTION
Hereditary breast cancer is estimated to account for approximately 10% of all breast cancer cases. In addition, an estimated 15-20% of those affected by breast cancer have a positive family history. Despite the fact that BRCA1 and BRCA2 are the two most significant genes in hereditary breast cancer predisposition, twenty years of analysis has highlighted the fact, that mutations in these two highly penetrant genes, are only present in approximately 20% of high risk families. Other genes, mutations in which are associated with high risk of breast cancer, were identified because of the strong association with familial cancer syndromes, in which breast cancer is one of the defining components. Technological advances in molecular biology and especially DNA sequencing, commonly designated as “Next Generation Sequencing – NGS” have aided in the concentrated efforts to identify new genes responsible for the missing heritability, allowing the application of this knowledge in the diagnostic setting.

AIM
The aim of this study was to investigate the extent and nature of mutations in 26 genes implicated in hereditary cancer predisposition in families of Romanian descent.

MATERIALS & METHODS
In total, 297 Romanian families have been analyzed by our group in the past three years. Genomic DNA was enriched for targeted regions of 26 genes involved in hereditary predisposition to cancer (ATM, BARD1, BLM, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM only intron 8, exon 9 and 3’UTR), FAM175A, MEN1, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, PALB2, PMS2, PTEN, RAD50, RAD51C, RAD51D, STK11, TP53, XRCC2). Sequencing was carried out using the Illumina NGS technology. Reads were aligned to the reference sequence (GRCh38), and sequence changes were identified and interpreted in the context of a single clinically relevant transcript. The presence of large genomic rearrangements was investigated by use of MLPA. All clinically significant observations were confirmed by orthogonal technologies.

RESULTS
In total, a pathogenic mutation was identified in 79 of the 297 families (26.6%) analyzed. Clinically significant mutations were identified in 17 of the genes included in the panel. The most commonly mutated genes in the Romanian population were BRCA1 and BRCA2, accounting for 50% of the mutations identified, followed by PALB2 (12%), CHEK2 (9.4%) and ATM, NBN and RAD50 which accounted for 3.5% of the mutations each. Of note is that 7 of the 79 affected families (8.8%) carried clinically significant mutations in two different genes.

CONCLUSIONS
Our results support the clinical significance of analysis of a panel of genes involved in hereditary cancer predisposition. In this series of patients, analysis of this panel allowed for the identification of 14% additional pathogenic variants. This is especially true in those cases where more than one pathogenic variant was identified.
Title: BRCA1/2 polymorphisms in Chinese breast cancer patients: Database from a national multi-center large-scale study

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Body: Background: To determine the characteristics of BRCA1/2 germline mutations and establish the BRCA1/2 mutations database of China, we launched a national multi-center study and detected the characteristics of BRCA1/2 germline mutations in a large cohort of Chinese people with breast cancer.

Methods: A total of 1725 Chinese women from 39 centers with breast cancer were enrolled in this study. Genomic DNA was extracted from the patient's saliva and be sequenced by next-generation sequencing for BRCA1/2 mutations.

Results: In total, 149 patients (9.21%) carried a BRCA1 or BRCA2 mutation (113 in BRCA1 and 117 in BRCA2) in this cohort and the mutation types is shown in table 1.

Table 1 BRCA1/2 mutation types in this cohort

<table>
<thead>
<tr>
<th>Mutation type</th>
<th>BRCA1 variants(%)</th>
<th>BRCA1 Mut a(%)</th>
<th>BRCA2 variants(%)</th>
<th>BRCA2 Mut a(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>113</td>
<td>70</td>
<td>117</td>
<td>112</td>
</tr>
<tr>
<td>Frameshift</td>
<td>37(32.7)</td>
<td>37(52.9)</td>
<td>24(20.5)</td>
<td>24(21.4)</td>
</tr>
<tr>
<td>Splicing</td>
<td>10(8.8)</td>
<td>10(14.2)</td>
<td>1(0.9)</td>
<td>1(0.9)</td>
</tr>
<tr>
<td>Nonsense</td>
<td>21(18.5)</td>
<td>21(30)</td>
<td>31(26.4)</td>
<td>31(27.7)</td>
</tr>
<tr>
<td>Missense</td>
<td>40(35.5)</td>
<td>2(2.9)</td>
<td>58(49.5)</td>
<td>56(20)</td>
</tr>
<tr>
<td>Inframe indel</td>
<td>2(1.8)</td>
<td>0(0.0)</td>
<td>1(0.9)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Intronic</td>
<td>3(2.7)</td>
<td>0(0.0)</td>
<td>2(1.8)</td>
<td>0(0.0)</td>
</tr>
</tbody>
</table>

a: pathogenic or likely pathogenic variants.

Also, we identified 72 novel BRCA mutations (31 in BRCA1 and 41 in BRCA2). BRCA1/2 mutation rate was 13.27 % in familial breast cancers, 10.73% in TNBC, respectively.

Table 2 BRCA1/2 mutation status in patients in different subgroups

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Total Number</th>
<th>BRCA1 mutations (%)</th>
<th>p value</th>
<th>BRCA2 mutations (%)</th>
<th>p value</th>
<th>BRCA1/2 mutations (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤45 years old</td>
<td>1059</td>
<td>39 (3.68%)</td>
<td>0.297</td>
<td>28 (2.64%)</td>
<td>0.136</td>
<td>67 (6.33%)</td>
<td>0.070</td>
</tr>
<tr>
<td>≤50 years old, ≥2 primary tumors</td>
<td>104</td>
<td>6 (5.77%)</td>
<td>0.798</td>
<td>5 (4.81%)</td>
<td>0.833</td>
<td>11 (10.58%)</td>
<td>0.626</td>
</tr>
<tr>
<td>Family history of cancer</td>
<td>309</td>
<td>22 (7.12%)</td>
<td>0.120</td>
<td>19 (6.15%)</td>
<td>0.112</td>
<td>41 (13.27%)</td>
<td>0.024</td>
</tr>
<tr>
<td>Triple negative, ≤60 years old</td>
<td>382</td>
<td>34 (8.90%)</td>
<td>0.004</td>
<td>7 (1.83%)</td>
<td>0.087</td>
<td>41 (10.73%)</td>
<td>0.252</td>
</tr>
<tr>
<td>Male breast cancer</td>
<td>12</td>
<td>0</td>
<td>0.967</td>
<td>0</td>
<td>1.000</td>
<td>0</td>
<td>0.640</td>
</tr>
</tbody>
</table>

Clinical characteristics such as family history, ER status, HER2 status, TNBC were closely related to BRCA mutations.
**Conclusion:** This study shows the BRCA mutations characteristics of Chinese people with breast cancer, and suggests the suitable crowd for BRCA detection.
2017 San Antonio Breast Cancer Symposium

Publication Number: P3-03-05

Title: Association between BRCA1 and BRCA2 mutations and survival in breast cancer patients according to molecular subtype

Olivier Tredan¹, Solene De Talhouet¹, Julien Peron², Alex Friedlaender³, Aurelie Vuilleumier³, Valeria Viassolo³, Aurelie Ayme³, Pierre O Chappuis³, Adrien Buisson², Valerie Bonadona¹, Alexandre Bodmer³ and S Intidhar Labidi-Galy³. ¹Centre Léon Bérard, Geneva, Switzerland; ²Hospices Civils de Lyon, Lyon, France and ³Geneva University Hospitals, Geneva, Switzerland.

Body: Background:
Germline mutations in BRCA1/BRCA2 genes increase the risk of breast and ovarian cancers. In ovarian cancer patients (pts) treated with platinum, BRCA1, and especially BRCA2, carriers have better survival than non-carriers. In breast cancer pts, BRCA1 carriers have higher response rate to neoadjuvant chemotherapy, however the benefit on survival remains unclear.

Patients and Methods:
A cohort study was conducted in France and Switzerland and consisted in 860 pts diagnosed with early breast cancer who underwent genetic screening for BRCA1/BRCA2 germline mutations and who received neo(adjuvant) chemotherapy. Disease-free survival (DFS) and Breast-cancer specific survival (BCSS) were analyzed according to BRCA1/BRCA2 mutations and breast cancer subtype (triple negative breast cancers (TNBC) vs non-TNBC).

Results:
One hundred and forty-three pts carried BRCA1 mutation; 88 had BRCA2 mutation; and 629 were non-carriers. Median age at diagnosis was 39 years; median follow-up was 4.8 years. Two hundred and fifty-three pts had TNBC (94 BRCA1, 14 BRCA2 and 145 non-carriers), and 607 had non-TNBC (49 BRCA1, 74 BRCA2 and 484 non-carriers). Among TNBC pts, BRCA2 mutations were associated with higher 5-years DFS (100% vs 71% for non-carriers and 80% for BRCA1 carriers; log-rank p=0.040), and BCSS (100% vs 78% for non-carriers and 92% for BRCA1 carriers; log-rank p=0.011). BRCA1 mutations were associated with higher 5-years DFS compared to non-carriers, but the association did not reach statistical significance (HR adjusted on nodal status=0.64; 95%CI 0.36-1.16, Wald test p=0.14). BRCA1 mutations were associated with an improved 5-years BCSS compared to non-carriers (HR adjusted on nodal status=0.38; 95%CI 0.16-0.87, Wald test p=0.022). In non-TNBC patients, BRCA1 or BRCA2 mutations were not associated with higher 5-years DFS (79% for BRCA1, 79% for BRCA2 carriers, and 80% for non-carriers; log-rank p=0.88) nor BCSS (94%, 92%, and 94% respectively; log-rank p=0.79).

Conclusions:
Among pts with TNBC, having BRCA germline mutation was associated with improved survival. In our cohort of TNBC pts, BRCA2 carriers did not present breast cancer relapse.
2017 San Antonio Breast Cancer Symposium

Publication Number: P3-03-06

Title: Telomerase polymorphism and breast cancer

Miriam M Alvares¹, Katherine S Rodrigues², João N Matos² and Diego M Oliveira². ¹Centro de Cancer de Brasília - CETTRO, Brasília, Distrito Federal, Brazil and ²Hospital Universitário de Brasília - HUB, Brasília, Distrito Federal, Brazil.

Body: Introduction:
Telomerase is an enzyme responsible for telomere maintenance in almost all human cancer cells, but generally not expressed in somatic ones. The minimum essential components of telomerase are the catalytic subunit, telomerase reverse transcriptase (TERT), and a non-coding RNA (TERC); TERT reverse transcribes telomere DNA using TERC as the template (1). Given the fundamental role of TERT in oncogenesis, polymorphisms of genes related to telomerase may influence the expression levels of this enzyme, influencing the host's susceptibility to tumor progression and metastasis [2,3]. Some studies have linked these polymorphisms with susceptibility and/or survival of patients with breast cancer [2,4,5,6]. In this work, we analyzed a region in the second intron of the gene TERT, located in chromosome 5p15.33, by sequencing, in order to find mutations associated with clinical aspects. The DNA of 65 patients with breast cancer were evaluated.

Methodology:
The population consisted of breast cancer patients who accepted to participate in the study, attended from March 2015 to September 2016 at a public hospital (HUB) and a private center for cancer treatment (Cettro®), both in Brasilia city – Brazil. Standard DNA extraction from blood samples was performed by dehydration and precipitation with saturated NaCl solution. The concentration and purity of the DNA were determined by spectrophotometry using the NanoDrop One equipment® (Themo Scientific, Madison, USA). The region of interest was amplified by conventional PCR. The primer sequences were: F: 5'-ATG CGA CAG TTC GTG GCT CA-3' and R: 5'-ATC CCC TGG CAC TGG ACG TA-3'. The sequencing was performed in the AB 3500 Genetic Analyzer® (Applied Biosystems). Data were analyzed by using software Chromas Lite ® (2.01, Technelysium Pty Ltd.) and the BLAST (NCBI - http://blast.ncbi.nlm.nih.gov/Blast.cgi). GraphPad Prism software version 7.02 was used for statistical analyzes.

Results and Conclusion:
A total of 65 patients were selected for DNA analysis. We identified a recurrent mutation (C>T) among them. The allelic frequencies were 0.79 for C and 0.21 for T. Only three patients had genotype TT, but 21 were heterozygous (CT). This polymorphism was significantly associated with tumor grade, the presence of T allele were implicated in more aggressive (grade 3) tumors (p=0.028, chi-square test). Those patients with genotype CT were at greater risk than others with CC genotype of having high grade (3) breast tumors (odds ratio = 6.13, 95% confidence interval 1.46, 25.74; relative risk 1.9, 95% confidence interval 1.02, 3.59).
Body: Introduction
The management of breast cancer (BC) patients who undergo mastectomy in the setting of 1-3 positive lymph nodes has been controversial. This retrospective Translational Breast Cancer Research Consortium study evaluated the molecular aberrations associated with locoregional recurrence (LRR) or distant metastasis (DM) compared to controls in an effort to identify molecular predictors associated with recurrence.

Methods/Materials
We identified 115 HER2 negative, therapy naïve, T 1-3 and N 0-1 BC patients treated with mastectomy and no post mastectomy radiation therapy from 1997 to present with available FFPE tissue blocks. The cohort included 32 patients with LRR, 34 with DM, and 49 controls (without recurrence) who were matched for stage, grade, hormone receptor status, age ≤ or > 50, chemotherapy receipt, and margin status. Matched primary and recurrent LRR samples were available for 3 patients. Hybrid capture next generation sequencing (NGS) of 142 cancer related genes and RNAseq were performed to identify DNA/RNA alterations associated with LRR or DM. The frequency of common alterations on NGS was compared with Fisher's exact test. Expression of each gene from mRNA-Seq was treated as an explanatory variable. Immunohistochemistry (IHC) was performed for PTEN, Ki-67 and cleaved caspase 3 (CC3). PTEN loss and percentage of Ki-67 and CC3 positive cells were compared between groups with Fisher's exact test and nonparametric methods, respectively.

Results
RNAseq was performed on 115 patients; there was no difference in RNA expression levels between the groups. DNA analysis was performed on 57 patients (17 LRR, 15 DM and 25 controls), NF1 mutation rate was significantly elevated in both the LRR (24%) and DM (27%) samples compared to controls 0%; (p=0.0070). The mitogen activated protein kinase (MAPK) pathway was significantly mutated in both LRR (47%) and DM (40%) samples compared to the controls 0%; (p<0.0001). There was no significant difference in the rate of alterations of the PI3K/Akt/mTOR pathway among the three groups. Of three patients with matched primary vs LRR samples, one had concordant mutations. The second patient had additional mutations in the LRR, including gain of a NF1 mutation. The third patient had complete discordance of mutations identified in primary and LRR and had gain of HER2 amplification, suggestive of a new primary. There was no significant association between the groups and the loss of PTEN expression or CC3 expression. There was a significant difference between Ki 67 positive cells in patients with LRR (mean 29%), DM (mean 26%) versus controls (mean 14%, p= 0.0011). HR+ patients were significantly more likely to have a positive PTEN, lower Ki-67 and lower CC3 expression, p=0.0004, p<0.0001, and p<0.0001 respectively.

Conclusions
In this matched cohort analysis, mutations in the MAPK pathway, specifically NF1, were associated with both LRR and DM, suggesting that alterations in this pathway are associated with a more aggressive tumor phenotype. However, there were no molecular features that discriminated between those likely to recur locally alone versus distantly. Further study is needed to validate these findings, and to determine whether targeting alterations in this pathway could decrease the risk of recurrence.
Body: Background: Despite a growing understanding of the somatic landscape of breast tumors from BRCA1 and BRCA2 mutation carriers, less is known about breast tumors from carriers of germline mutations in other homologous recombination and DNA repair pathway genes such as ATM, CHEK2, and PALB2.

Methods: We identified 44 clinically annotated breast cancer cases that included carriers of germline mutations in BRCA1 (n=9), BRCA2 (n=9), ATM (n=5), CHEK2 (n=7), and PALB2 (n=6) from the Hereditary Cancer Risk Program at BUMC. Sporadic breast cancers cases (n=8) were also collected. Genomic DNA and RNA were extracted from macro-dissected FFPE tumor sections, adjacent normal FFPE tissue, along with constitutional genomic DNA from blood. Expanded whole exome sequencing (WES) was performed on normal/tumor pairs and RNA-seq from tumors for each case. Bioinformatics analysis was performed using industry standard methods for somatic characterization.

Results: All germline mutations were confirmed by WES. Somatic mutational analysis and copy number profiling from WES revealed the greatest similarities among BRCA1 and CHEK2 carriers. As expected, TP53 mutations were found in 8 of 9 BRCA1 carriers as all were triple negative subtype. We also detected somatic TP53 mutations in tumors from 4 of 7 CHEK2 carriers. Somatic TP53 mutations were found in only 1 of 7 BRCA2 tumors and 1 of 4 PALB2 tumors tested. Furthermore, BRCA1 and CHEK2 tumors showed trends of having higher mutation burden. Analysis of copy number BRCAness demonstrated stronger similarities between BRCA1, ATM, CHEK2, and PALB2 tumors. BRCA2 tumors were unique with fewer events and characterized by specific amplifications including 11q23 (CCND1) and 17q23 (BRIP1). Hierarchical clustering of RNA-seq data revealed strong clustering of BRCA1 tumors compared to all other tumors, predominantly attributed to breast cancer subtype. Furthermore, pathway analysis of genes that distinguish BRCA1 mutation positive versus non-BRCA mutated tumors showed strong correlation to pro-inflammatory and immune pathway signatures.

Conclusions: Molecular analysis of 44 breast cancers from individuals with inherited predisposition to breast cancer via BRCA1, BRCA2, ATM, CHEK2, and PALB2 germline mutations demonstrated strongest somatic similarities between BRCA1 and CHEK2 tumors although all BRCA1 were TNBC and all CHEK2 tumors were ER positive. Marked differential gene expression differences in RNA expression patterns were observed in BRCA1 mutation carriers compared with all other groups analyzed. Our study is among the first to interrogate the profile of non-BRCA mutated hereditary breast cancers.
Body: Background The human epidermal growth factor receptor 2 (ERBB2) can be altered by somatic mutations (with/without ERBB2 amplification) that likely drive tumorigenesis. Here, we present the first study that thoroughly investigates the behavior of patients with ERBB2 mutated tumors in a large unselected cohort of metastatic breast cancer (MBC) patients.

Patients and Methods We included retrospectively all MBC patients with sufficient tumor material available, independent of hormone receptor or ERBB2 amplification status, diagnosed between 2000 and 2015 at the Multidisciplinary Breast Center of University Hospitals Leuven. Genomic DNA extracted from primary breast tumors was subjected to deep targeted re-sequencing using an assay covering 5 exons (exons 8, 17, 19, 20 and 21) known to accumulate ERBB2 hotspot mutations. Clinical characteristics, treatment response and outcomes (excluding bilateral BC) were compared between patients with and without ERBB2 mutations.

Results We established and validated a research use only next-generation sequencing assay across five exons of ERBB2. Somatic ERBB2 mutations with an allelic frequency of ≥5% in at least one independent analysis, were observed in 1.8% (13/721) of MBC patients. MBC patients with ERBB2 mutant primary tumors appear to have similar patient and tumor characteristics (TNM stage, grade, and receptor and HER2 status) compared to non-mutant patients and ERBB2 mutations occur in all molecular subtypes (7 ER+/HER2-, 4 HER2+, 2 triple negative). However, we see a small trend towards enrichment in the invasive lobular carcinomas (ILC) in the ERBB2 mutant patients vs. the non-mutant patients (4/13 (31%) vs. 97/695 (14%), p=0.086). ER+ patients with an ERBB2 mutant tumor who received first line aromatase inhibitor (AI) (n=3) had a worse median time to progression (TTP) compared to non-mutant patients (n=156) (TTP 103 vs. 311 days, p=0.04), AI use in any line had also a worse TTP (n=8 vs. 376; TTP 74 vs. 213 days, p=0.006). TTP was not significantly different for other standard therapies, but numbers were small. Median distant disease free survival (DDFS) defined as time from primary diagnosis to first metastasis, was slightly shorter for ERBB2 mutant tumors (n=9 vs 514; DDFS 1.4 vs. 2.9 years, p=0.066). However, in ER+/HER2- ERBB2 mutant tumors, median DDFS was significantly shorter in non-mutant patients (n=5 vs. 328; DDFS 0.8 vs. 4.0 years, p=0.02). Median overall survival (OS) after diagnosis of MBC was numerically lower for mutant patients in all subtypes vs. non-mutant patients (n=11 vs. 669; OS 1.1 vs. 2.3 years, p=0.457), and in ER+/HER2- disease (n=6 vs. 431; OS 1.0 vs. 2.9 years, p=0.07).

Conclusion In our large MBC cohort, patients with ERBB2 mutant tumors appear to have similar tumor and patient characteristics as ERBB2 non-mutant tumors. ERBB2 mutations do not seem to occur more or less often in specific breast cancer subtypes except for a slight increase for ILC. In ER+/HER2- MBC, patients with ERBB2 mutant tumors appear to be adversely prognostic; having a shorter DDFS after early breast cancer treatment, and in metastatic disease, they respond worse on AI and have a trend for worse OS compared to non-mutant cases.
Title: Germline and somatic mutation status in tissues from BRCA1/2 carriers

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Body: Background – aim: In carriers of BRCA1/2 pathogenic mutations (mut), it is expected that the germline mut is present in all tissues, particularly in normal; the somatic mut status in normal tissues from these patients is usually not addressed. We investigated the mut status in normal and tumor tissues in a real-life cohort of BRCA1/2 carriers who underwent prophylactic surgery.

Methods: All 53 women had known BRCA1/2 germline mut that had been assessed independently; 42 had previous cancer manifestation (PCM); all had prophylactic mastectomy; 22 had prophylactic hystero-salpingo-oophorectomy. By using a 60-gene NGS panel, we examined the mut status of 231 samples, 39 peripheral blood and 192 paraffin tissues (FFPE: 46 tumors, out of which 43 breast; 97 normal breast [NB]; 49 normal ovary and salpinx [NGYN]). Germline mut status was interrogated in tissues with the above panel, Sanger sequencing and a multiplex PCR protocol for large exonic deletions, along with extensive FFPE DNA quality control (QC) to exclude false negatives.

Results: Eight patients carried germline BRCA2 and 45 BRCA1 mut (29 in the BRCT-domain; 31 substitutions/indels). We identified somatic mut in 85% of the tumors and in 64% of the normal samples; mut were found significantly more often (p=0.003) and in higher numbers (p<0.001) in NGYN than in NB. In NB and NGYN, top 3 genes with somatic mut were BRCA2 (28%), BRCA1 (17%), TP53 (7%). In tumors, somatic mut were most frequent in TP53 (49%; p<0.001) and BRCA1 (38%; p=0.039). Among all tissue types, the 5 tumors post-neoadjuvant treatment had the highest and NB the lowest mut load (p=0.001). In NB and NGYN, mut load was not affected by PCM or BRCA1 mut domain but it was higher in BRCA1 vs. BRCA2 carriers (p=0.027) and in those with BRCA1 substitutions/indels vs. exon deleting and skipping mut (p<0.001). In tumors, germline BRCA1 substitutions/indels were associated with higher mut load (p=0.014). We validated germline mut status in all blood samples and in 111 tissue samples that passed FFPE DNA QC from 40 patients. The germline mut was not found in 14 samples (4 breast tumors; 3 NB; 7 NGYN) from 10 (25%) patients, all BRCA1 carriers, 9 with germline mut in the BRCT-domain. The only non-BRCT domain germline mut that was lost in one breast tumor, p.V1234fs, was replaced by the R1751* (validated), again in the BRCT domain. In normal tissues, those with lost germline mut had significantly less somatic mut compared to those with preserved germline mut (p<0.001).

Conclusions: In BRCA1/2 carriers, somatic mut in BRCA genes and TP53 are present in normal breast and GYN tissues, more frequently in the latter, and seem associated with the mutated gene and with the type of mut in the germline. The mut status of normal breast tissue does not seem to be affected by neoadjuvant chemotherapy for breast cancer. The observed BRCA1 germline mut loss, particularly in normal tissues, may be approached as a negative selection for the inherited mut; similarly to the described germline mut reversion after chemotherapy, tissues may react to deleterious effects of haploinsufficiency, which needs functional validation.
Title: Outliers—extreme long-term survivors with metastatic breast cancer

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Body: Breast cancer has an extremely heterogeneous clinical course, but the specific causes for this diversity are largely unknown. Understanding the basis for extreme long-term survival with incurable cancer can be of value for prognosis or possible interventions if they arise from modifiable factors.

Methods: We identified patients at our institution with persistent and distant metastases who are alive greater than 5 years from initial diagnosis, and evaluated the clinical characteristics of this population. Additionally we evaluated the published METABRIC cohort of patients who died of cancer to compare patients with greater than median survival (late) to those who died with less than median survival (early). In doing so, we evaluated the non-exclusive alternative hypotheses that long-survival is dictated by cancer genomes versus by host immunity. To do this, we grouped somatic mutations into 11 common pathways and compared the likelihood of being present in the tumors causing early versus late death, when stratified for hormone receptor [HR] status. To evaluate the effect of immune infiltration, we used gene expression profiles using the CIBERSORT algorithm (Nat Methods 12:453, 2015).

Results: We identified 53 individuals who are alive with metastatic breast cancer greater than 5 years from the original diagnosis. The four longest-term survivors were diagnosed with breast cancer in 1978, 1978, 1979, and 1980 and developed metastasis in 1982, 2007, 1996, and 2000 respectively. The median time to metastasis was 4.5 years. Of these, 80% had ER and/or PR-positive breast cancer, 9% triple negative and 18% had HER2+ disease. The longest-term survivors tend to have well-differentiated histology. Analysis of the METABRIC data, demonstrated, as expected, that long survival correlates with hormone-receptor-positive disease. Among 647 subjects who died of cancer, 192 were HR-negative (median survival 36 mo.) and 455 were HR-positive (median survival 76 mo.). Among HR-positive there were no statistical differences in genome profiles between long-versus-short survivors. By contrast, among HR-negative tumors, mutations PI3K-pathway genes and microenvironment maintenance were associated with longer survival (p=0.041 and p=0.047, respectively). CIBERSORT was used to provide quantitative estimates of infiltrating tumor cells, allowing patient groups to be divided by quartiles. However, no significant differences were found in survival among quartiles in T-cell infiltration, in T-cell subsets (activated CD4 memory, CD8, and Tregs) or in monocyte/macrophage subsets.

Conclusions: Some patients live for extended periods of time with metastatic breast cancer. Long-term survival of breast cancer can be explained, in part, by the cell-autonomous features of the tumor, including HR status and genes involved in PI3K signaling and microenvironment maintenance, but no major differences were found in immune infiltrates. Although these data are representative of longer-than-median survivors, extreme long-term survivors may have additional unique features in genomic and immune characteristics.
Title: Genomic analysis of cancers arising in breasts with different mammographic density

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Body: Background: High mammographic density (MD) is a known risk factor for breast cancer, but it is currently unknown whether this altered microenvironment leads to the development of genomically different tumours.

Method: We used lifepool, an Australian prospective population-based cohort of over 53,000 women currently in a mammographic screening program, to identify >1000 cases of breast carcinoma (in situ and invasive breast cancer) for analysis. Pathology reports, detection modality (screen-detected/interval), MD data (AutoDensity, Nickson et al., 2013 DOI:10.1186/bcr3474), germline DNA and tumor tissue were available for a subset of cases. Tumours were classified into quintiles, depending on the relative MD ranking of the most recent normal mammogram. Tumour DNA was analysed using Affymetrix OncoScan MIP arrays or using a targeted Agilent SureSelect and Illumina sequencing panel of 105 genes relevant to breast cancer. Copy number and mutation data were compared between tumours from the highest (high-MD) and lowest (low-MD) quintiles of MD.

Results: Preliminary analysis found no significant differences in clinico-pathological features between tumours from breasts with high-MD (n=38) and low-MD (n=35). Genomic analysis of 8 high-MD tumours and 8 low-MD tumours identified chromosome 15 loss as being significantly enriched in low-MD tumours. Only Low-MD tumours to date contained TP53 mutation (3/8). Data will be presented from at least an additional 9 low-MD and 15 high-MD tumours currently undergoing genomic assays.

Conclusion: When complete, our data will be the first to evaluate the somatic genetic events in tumours arising in low-MD and high-MD breasts. Although our cohort to date is small, it may be possible that tumours from extremes of MD have different genomic characteristics.
Title: Novel genetic features associated with 8p11-p12 amplification in breast carcinoma

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Body: Background: Genome instability contributes to the neoplastic phenotype by promoting gene loss and duplications, which in turn can have a detrimental effect on patient outcome by inactivating tumor suppressor genes or hyperactivating oncogenes. In breast carcinoma, DNA amplification of the 8p11-p12 genomic region has been associated with tumor progression and poor prognosis. The aim of this study was to characterize recurrent genetic features (other than DNA amplification) associated with 8p11-p12 amplification in breast carcinoma.

Methods: DNA copy number profiling data for 229 primary invasive breast carcinomas (corresponding to 185 patients diagnosed in Western Sweden between 1988 and 1999) were evaluated to identify 8p11-p12 amplified cases. Illumina paired-end whole transcriptome sequencing (RNA-seq) and whole-genome SNP genotyping were subsequently performed on 23 breast carcinomas harboring high-level regional 8p11-p12 amplification to characterize recurrent genetic variants (SNPs and indels), expressed gene fusions, gene expression profiles and allelic imbalances. The 23 samples were stratified into the molecular subtypes, resulting in 16 Luminal B/HER2-, two Luminal B/HER2+, four HER2/ER-, and one Basal-like sample. The Cancer Genome Atlas (TCGA) RNA-seq data for 10 primary breast carcinomas lacking the 8p11-p12 amplicon (SNP segmented mean < 0.4) were used as controls. Gene fusions were validated using dual-color fluorescence in situ hybridization (FISH) with co-hybridized biotin-16-dUTP and dioxigenin-11-dUTP labeled bacterial artificial chromosome (BAC) probes.

Results: Here, we report that despite the high number of gene fusions (133±31 (±SEM)) and exonic variants (411±16) identified per tumor, few gene fusions (n=46) and exonic variants (n=11) spanned the 8p11-p12 genomic region. Gene fusions predominantly contained at least one fusion partner spanning non-coding RNAs (ncRNAs; 86%), in particular MALAT1, which is induced by estrogen and of prognostic value in breast cancer. The majority of fusion breakpoints were associated with DNA copy number gains and losses, as well as, extensive intratumoral heterogeneity for specific fusion events. Intriguingly, novel 8p11-p12 amplification-specific genetic variants (HIST1H1E frameshift insertion, UQCRHL nonsynonymous SNV, MTUS1 frameshift insertion, NPIPA5 frameshift deletion) were identified that also resulted in mutation-dependent changes in gene expression levels.

Conclusions: Taken together, these findings have provided further insight into the genetic landscape of 8p11-p12 amplified breast carcinomas, including novel gene fusions and genetic variants. However, further studies are required to develop effective strategies to target 8p11-p12 amplification in breast carcinoma.
2017 San Antonio Breast Cancer Symposium

Publication Number: P3-04-08

Title: Genomic analysis of breast papillomas

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Body: Background: Papillomas are often found co-existing with breast carcinoma yet they are not considered to be a true precursor of the disease. Previous studies have shown that some cases may carry copy number alterations (CNA) or mutations in AKT1/PIK3CA (Troxell et al., 2010, Modern Pathology 23: 27-37) suggesting this lesion may have malignant potential. To date, a detailed study of both pure papillomas (not associated with cancer) and those seen in the same breast as a carcinoma has not been undertaken. Therefore, we set out to investigate the molecular changes associated with this lesion and whether papillomas can be clonally related to synchronous breast carcinoma.

Method: Papilloma cases were identified from a hospital database and independently reviewed by consultant pathologists followed by micro-dissection of formalin-fixed paraffin-embedded tumour tissue and DNA extraction. For CNA detection either Affymetrix Molecular inversion Probe (MIP) 330K arrays were used or low-coverage whole genome sequencing using 5-10 ng of DNA (Kader et al., 2016, Genome Medicine 8: 121) where there was insufficient DNA for MIP arrays. We applied either of these 2 methods to 24 cases of pure papilloma as well as 20 papilloma with synchronous ductal carcinoma in situ (DCIS) and/or invasive ductal carcinoma (IDC). Additionally, targeted exon sequencing of breast cancer driver genes was performed for a subset of cases.

Results: Among the pure papillomas 31% (5/16) showed CN change with, the most frequent change being 16q loss (2/16). Of the papillomas synchronous with DCIS/IDC analysed to date, 2/5 were shown to be clonal with the co-existing carcinoma. Final CNA analysis will be presented for 24 pure papilloma cases and 20 synchronous cases. Targeted sequencing revealed that all for pure papillomas analysed to date harboured somatic mutations in PIK3CA (3/4 cases) and PIK3R1 (1/4,) suggesting that most papillomas are driven by alterations in the PI3-kinase/AKT pathway. The final sequencing data to be presented will include an additional 5 pure papillomas and 10 synchronous cases.

Conclusion: Our observation that 40% of papillomas are clonal to breast carcinoma suggests that DCIS or IDC can arise from a common ancestor as co-existing papillomas, however, most papillomas co-existing with carcinoma are likely to be independent in our cohort.
2017 San Antonio Breast Cancer Symposium

Publication Number: P3-04-09

Title: Integrated analysis of genetic variations in Chinese breast cancer from a single institution

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Body: To investigate the characteristics of somatic mutation or copy number variations in Chinese breast cancer, tumor tissues from 105 patients diagnosed at age from 26 to 81 (median age 48) were assessed by next-generation sequencing technology using a customized panel, including 33 genes of putative tumor suppressors or oncogenes. At least one genetic alteration (including mutations, copy number variations and fusion genes) was observed in 99/105 (94%) samples. Similar to the previous report in TCGA dataset, TP53 (49%) and PIK3CA (43%) were the most frequently mutated genes, which occurred in a significant mutual exclusive manner (p<0.05). Three genes including MYC copy number amplification (35%), FGFR1 (19%) and GATA3 mutation (16%) were altered at the frequency of >10% in our dataset, in which the occurrence of MYC amplification was higher than the TCGA cohort (22%, p<0.05). Importantly, we identified four fusion genes of FGFR1 including one previously reported (TACC1-FGFR1) and three novel fusion ones (MIR1268A-FGFR1, LZTS1-AS1-FGFR1, and LINC01605-FGFR1). Unlike the high prevalence of CCND1 amplification (17%) and CDH1 mutations (13%) in TCGA dataset, genetic variations of CCND1 and CDH1 in our study occurred at a low frequency with 2% and 4%, respectively (p<0.05). In addition, we also identified three novel ESR1 mutations (ESR1 G74R, D230H and M250T) in the untreated patients with early breast cancer. Furthermore, nonnegative matrix factorization (NMF) clustering of genetic variations revealed five distinct molecular classes in our dataset. NMF class I was characterized by a high rate of HR+/ERBB2- tumors (80%) and genetic alterations of FGFR1 (100%). A gain of ERBB2 gene was observed in 93% of NMF class II along with TOP2A amplification in 57% of HR+/ERBB2+ tumors. NMF class III was characterized by a high rate of HR+/ERBB2- (95%) and GATA3 mutations (75%) without TP53 mutation. The characteristics of NMF class IV were the high rate of PIK3CA mutations (95%) and HR+/ERBB2- tumors (75%) along with low rate of TP53 mutations. More HR-/ERBB2- tumors (39%) were observed in NMF class V with a high rate of MYC amplification (82%) and TP53 mutation (89%). Further analysis in the TCGA cohort revealed the patients in NMF class V had the shorter survival time than other clusters. Collectively, we identified several novel genetic variations and generated a preliminary profile of somatic genetic aberrations that could classify Chinese breast cancer in this study, and may represent novel therapeutic targets for molecular subsets of breast cancer.

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2017 San Antonio Breast Cancer Symposium

Publication Number: P3-04-10

Title: Analysis of somatic transposable element insertions in a breast cancer genome

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Body: Introduction: More than 50% of the human genome consists of transposable elements (TE). However most TEs are inactive and remain relatively stable among different human populations. However, some classes of retrotransposons consisting of Alu, SVA, ERV and L1s are active and believe to be involved in cancer. Commonly used bioinformatics pipelines of whole genome sequencing (WGS) data do not currently analyze for somatic TE insertions, and maybe missing an important class of somatic mutations. As a proof-of-concept, we analyzed the HCC1143 triple-negative breast cancer cell line along with a matched normal control to analyze for the presence of somatic TEs.

Methods: WGS data of the HCC1143 cell line along with the matched lymphoblastoid control cell line, HCC1143BL, were downloaded from the NCI GDC Commons. We used discordant read-pair (only one paired-end read matches to the reference genome) and split-read (part of a read matches to the reference) analysis to detect novel somatic TE insertion sites in genes known to play important roles in cancer proliferation.

Results: Most TE insertions are heterogenous in nature such that they insert only in a small subset of the cells. Therefore by comparing the number of split reads and discordant reads to the total number of reads the heterogeneity of the insertion can be inferred. The discordant and split read analysis revealed about 30 high confidence hits. A subset of the high confidence hits were validated experimentally using PCR amplification and gel electrophoresis. About 85% of the hits were successfully validated. A subset of the validated hits known to be relevant to breast cancer have been shown in Table 1.

Table 1 - Validated somatic TE insertion locations in HCC1143 cell line

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
<th>Chromosome number</th>
<th>Start position</th>
<th>Stop position</th>
<th>% heterogeneity in tumor line = (split_reads + discordant_reads)*2 / total_reads</th>
<th>% heterogeneity in blood line = (split_reads + discordant_reads)*2 / total_reads</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBRM1</td>
<td>Chromatin remodeling</td>
<td>3</td>
<td>52662648</td>
<td>52663550</td>
<td>100</td>
<td>1.1</td>
</tr>
<tr>
<td>GLIS3</td>
<td>Overexpressed in TNBC/involved in Wnt pathway</td>
<td>9</td>
<td>4236979</td>
<td>4237291</td>
<td>40</td>
<td>2.5</td>
</tr>
<tr>
<td>XPR1</td>
<td>Receptor of murine leukemia viruses</td>
<td>1</td>
<td>180857399</td>
<td>180857714</td>
<td>25</td>
<td>3.2</td>
</tr>
<tr>
<td>CSMD1</td>
<td>Tumor suppressor gene</td>
<td>8</td>
<td>4452610</td>
<td>4453043</td>
<td>23.52</td>
<td>0</td>
</tr>
<tr>
<td>TMEM181</td>
<td>Involved in tamoxifen response</td>
<td>6</td>
<td>159053267</td>
<td>159053825</td>
<td>4.71</td>
<td>0</td>
</tr>
</tbody>
</table>

Discussion: Using the approach described above, we were able to detect somatic TEs within several genes known to be involved in cancer. PBRM1, a gene involved in chromatin remodeling, was found to have several exons replaced with a TE insert. Other genes such as CSMD1 is a known tumor suppressor gene, and TMEM181 which is involved in tamoxifen response. These results suggest that TE insertions may be involved in cancer biology. Therefore, TE insert prediction can serve as useful biomarkers for early detection and have precision medicine implications in the treatment of breast cancer. Analyses in additional breast cancer patient genomes is currently underway.
Title: Implications of somatic TP53 and PIK3CA mutations in patients with metastatic breast cancer who underwent germline BRCA testing

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Body: Background: TP53 and PIK3CA are the most frequently mutated genes in breast cancer. However, there is limited data evaluating the impact of somatic mutations in TP53 and/or PIK3CA patients (pts) with metastatic breast cancer (MBC) who have undergone germline testing for BRCA mutations. Here, we report the frequency of somatic TP53 and PIK3CA mutations in MBC pts who previously underwent germline BRCA (gBRCA) testing and evaluate their impact on long-term outcomes.

Methods: We identified pts with MBC from our prospectively maintained high risk genetics database who underwent gBRCA testing and had somatic mutation testing performed for TP53 and/or PIK3CA through hotspot sequencing of single genes or as part of a next generation sequencing panel. Univariable logistic regression was used to evaluate associations between clinicopathological characteristics, including gBRCA status, and the presence of somatic TP53 and PIK3CA mutations. Overall survival (OS) was defined as the time from diagnosis to death from any cause. Multivariable cox regression analysis was used to identify independent predictors of OS.

Results: 104 pts met all inclusion criteria. Somatic mutations in TP53 and PIK3CA were found in 46% (39/84) and 23% (24/104) of tested pts, respectively. Associations between clinicopathological characteristics and somatic TP53 and PIK3CA mutation status are summarized in Table 1. Pts with hormone receptor positive (HR+) disease were less likely to have TP53 mutations (odds ratio [OR]: 0.28, 95% confidence interval [CI]: 0.11-0.70, p=0.007) but more likely to harbor PIK3CA mutations (OR: 3.27, 95% CI: 1.11-9.62, p=0.031). There were no significant associations between gBRCA mutation status and somatic TP53 or PIK3CA mutations. On multivariable analysis, somatic TP53 mutations (adjusted hazard ratio [aHR]: 1.98, 95% CI: 1.10-3.58, p=0.023) and de novo metastatic disease (aHR=2.88, 95% CI: 1.26-6.58, p=0.012) independently predicted poorer OS, while HR+ disease (aHR: 0.40, 95% CI: 0.21-0.74, p=0.004) and HER2+ disease (aHR: 0.33, 95% CI: 0.13-0.83, p=0.019) were significantly associated with improved OS. PIK3CA mutations (aHR: 1.20, 95% CI: 0.59-2.44, p=0.62) and gBRCA mutations (aHR: 0.78, 95% CI: 0.33-1.83, p=0.57) did not significantly impact OS in this study.

Conclusion: In this study, somatic TP53 mutations independently predicted worse OS in pts with MBC after adjusting for significant covariates, including gBRCA mutation status. These findings should be validated in a larger cohort of pts.

Table 1: Association between patient characteristics and somatic TP53 and PIK3CA mutation status

<table>
<thead>
<tr>
<th></th>
<th>TP53</th>
<th>PIK3CA</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TP53 mutant</td>
<td>TP53 wild type</td>
<td>PIK3CA mutant</td>
<td>PIK3CA wild type</td>
</tr>
<tr>
<td></td>
<td>(n=39)</td>
<td>(n=45)</td>
<td>(n=24)</td>
<td>(n=80)</td>
</tr>
<tr>
<td>Median Age – years</td>
<td>37 (33-47)</td>
<td>39 (36-44)</td>
<td>0.937</td>
<td>39 (34-46)</td>
</tr>
<tr>
<td>(Interquartile Range)</td>
<td></td>
<td></td>
<td></td>
<td>38 (34-43)</td>
</tr>
<tr>
<td>gBRCA mutant – n(%)</td>
<td>3 (8)</td>
<td>11 (24)</td>
<td>0.051</td>
<td>1 (4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15 (19)</td>
</tr>
<tr>
<td>ER/PR positive - n(%)</td>
<td>17 (44)</td>
<td>33 (73)</td>
<td>0.007</td>
<td>19 (79)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>43 (54)</td>
</tr>
<tr>
<td>HER2 positive - n(%)</td>
<td>8 (21)</td>
<td>6 (13)</td>
<td>0.382</td>
<td>7 (29)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10 (13)</td>
</tr>
<tr>
<td>De Novo Metastatic –</td>
<td>6 (15)</td>
<td>8 (18)</td>
<td>0.769</td>
<td>3 (13)</td>
</tr>
<tr>
<td>n(%)</td>
<td></td>
<td></td>
<td></td>
<td>16 (20)</td>
</tr>
<tr>
<td>Visceral Disease – n</td>
<td>23 (59)</td>
<td>26 (58)</td>
<td>0.912</td>
<td>15 (63)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>47 (59)</td>
</tr>
</tbody>
</table>
Title: Immune and transcriptional signatures of dendritic cell (DC) vaccination combined with chemotherapy in locally advanced, triple-negative breast cancer (TNBC) patients

A Karolina Palucka¹, Lee K Roberts², Sandra M Zurawski², Jessica Tarnowski², Jacob Turner², Xuan Wang², Derek Blankenship², Jennifer L Smith³, Maren K Levin², Jennifer P Finholt², Susan B Burkeholder², Roxana Timis², Luz Stella Muniz², Tuoc Dao³, Michael Grant³, Jacques Banchereau¹, Gerard Zurawski², Virginia Pascual⁴ and Joyce A O’Shaughnessy³.

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BACKGROUND: Women with TNBC who do not achieve a pathologic complete response (pCR) with preoperative (preop) chemotherapy have a high risk of recurrence and death from BC. Immunotherapy is an attractive strategy as human BCs can be immunogenic, and enhancing the immune effector function may augment the cytotoxic effects of standard therapies.

CLINICAL TRIAL: Following IRB-approved informed consent, 10 pts with locally advanced TNBC received preop dose-dense doxorubicin/cyclophosphamide (AC) followed by paclitaxel and carboplatin (TCb) chemotherapy, combined with antigen-loaded (TNBC antigens: Cyclin B1, WT1, and control viral antigens: CEF) autologous monocyte-derived DC vaccinations administered intratumorally and subcutaneously. DCs were generated with GM-CSF and type I interferon, loaded with antigen in the form of long peptides and activated with innate ligands (LPS and Clo75) and CD40 ligand. Vaccines were given at 4 time points prior to definitive surgery, and 3 times post-surgery, pre- and post-radiation therapy (RT). Safety was the primary study endpoint, and pCR rate in breast and axilla was a secondary endpoint. Correlative studies included assessment of immune response via ELISpot and transcriptional profiling of blood samples collected over time.

RESULTS: All pts received the 4 vaccines during preop chemotherapy, and 7/10 received all 7 vaccines. At the time of definitive surgery, 4 pts achieved a pCR, 3 pts had macroscopic residual disease in the breast and axillary lymph nodes, and 3 pts had residual cancer burden scores of 1. As of June 1, 2017, all pts have been in follow-up for at least 1 year s/p completion of all vaccines, and 7/10 patients have no evidence of disease.

To assess immune signatures with IFN-γ-ELISpot, PBMCs from baseline (BL) and several time points during vaccine treatment were cultured with control peptides or with peptide libraries covering vaccine antigens. Using a linear mixed model to account for repeated and missing observations we found statistically significant (α = 0.05) increases in Cyclin B1, WT1, and CEF ELISpots in at least 1 time point post-DC vaccination and in follow-up. Compared to BL, Cyclin B1 and WT1 increased at 3 day pre-RT in 8/10 and 7/10 pts, respectively. To assess transcriptional signatures, a linear mixed model was utilized to determine statistically significant differences in fold-change over time compared to the BL and healthy controls. Modular analysis of differentially expressed transcripts at BL revealed downregulation of transcripts related to the monocyte lineage in 7/10 pts. Longitudinal analysis revealed profound transcriptional changes during AC with downregulation of lymphocyte modules and upregulation of innate and inflammation modules. While the latter ones have normalized during TCb and follow-up, T cell module remained substantially downregulated throughout treatment and follow-up.

CONCLUSIONS: Combination of preop chemotherapy and intratumoral and subcutaneous autologous DC vaccination is safe in locally advanced TNBC pts and is linked with profound changes in immune transcription signatures and with expansion of antigen-specific immune responses that can be detected in IFN-γ ELISpot.
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Title: Immune profiling of paired primary and recurrent triple negative breast cancer

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Body: Background: Triple negative breast cancer (TNBC) is a heterogeneous disease with several molecular subtypes: basal-like1 (BL-1), basal-like 2 (BL-2), mesenchymal (M), and luminal androgen receptor (LAR). Molecular evolution of TNBC through chemotherapy selection pressure is well recognized but poorly understood. In addition, approximately 20% of TNBCs respond to PD-1 or PD-L1 inhibitors. It has been observed that heavily pre-treated patients may not respond well to immunotherapy. This study was carried out to perform immune profiling of paired primary and recurrent TNBC. Here we report the result of the first 10 paired tissue pilot analysis.

Methods: Twenty specimens were identified through an IRB-approved protocol via the City of Hope Biospecimen Repository (2002-2015). Two brain and one bone metastasis specimens were not included due to technical difficulty. Formalin-fixed paraffin embedded (FFPE) sample blocks were cut into 5-mm thick slides and labeled with the following antibodies: CD4, CD8, CD3, FOXP3, CD20, CD33, Pan-CK, and PD-1 using the multiplex IHC opal method. Image acquisition and cell counting were carried out using PerkinElmer Vectra automated quantitative pathology imaging system and inForm software analysis (PerkinElmer, Waltham, MA). mRNA expression profiling was performed using Affymetrix Human Genechip 2.0. Raw data were normalized and processed using Expression Console. Using Vanderbilt TNBC sub-classification tool, we have sub-classified the 20 primary and recurrent TNBC specimens. Tumor mutation burden (TMB) was generated through FoundationOne® platform.

Result: A total of 17 samples were analyzed (M, 5; LAR, 3; BL-1, 4; BL-2, 5). M-subtype had a significantly lower tumor-infiltrating CD3+ T cells (p=0.005), CD8+ T cells (p=0.024), CD4+ T cells (p=0.065) and CD4+FOXP3+ Treg cells (p=0.054), irrespective of the site of metastasis. CD20+ B cells were particularly enriched in BL-1 subtype (p=0.0013, 23.5% of 17 samples). Of 17 samples, 8 had TMB. Seven had low TMB (<10 mut/Mb) and one had intermediate TMB (11 mut/Mb, LAR subtype). The tumor with intermediate TMB had the highest quantity of tumor-infiltrating CD3+ T cells, CD8+ T cells, CD8+PD1+ T cells, and CD4+FOXP3+PD1+ Treg cells compared to the 7 tumors with low TMB. Compared with recurrent tumors, primary tumors had a significantly higher percentage of tumor-infiltrating T cells (TIL). To validate multiplexed IHC results, these samples were evaluated by a licensed pathologist at City of Hope using the International TILs Working Group 2014 guidelines, and there was a good correlation between percent of TILs and CD3+ T cells by IHC approach.

Conclusion: To our knowledge, this is the first study linking tumor immune cell profiles with the TNBC 4 subtypes. Distinctive immune cell patterns were observed among 4 TNBC subtypes. M subtype had significantly lower TILs, which may indicate poor response to checkpoint inhibitors. Further analysis of a total of 50 paired TNBCs is currently underway.

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Title: Metaplastic breast cancers: Genomic profile, mutational burden and TILs

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Body: Background: Metaplastic breast cancers (MPBC) are rare, typically triple negative aggressive tumors composed of both, adenocarcinoma and metaplastic elements. Recent evidence that TNBC and MPBC can respond vigorously to immune checkpoint inhibitor therapy (Adams et al, ASCO 2017 and npj Breast Cancer 2017) has prompted the following comprehensive genomic profiling (CGP) and histopathologic assessment of tumor infiltrating lymphocytes (TIL) designed to uncover potential biomarkers of immunotherapy response for MPBC, including mutational burden, Microsatellite Instability (MSI) status and gene amplification of 9p21.4 (or CD274, which includes the PD-L1 locus).

Methods: 12,214 locally aggressive, relapsed and metastatic breast malignancies (mBM) were subjected to CGP using DNA extracted from 40 µm of FFPE sections and adaptor ligation-based libraries to a mean coverage depth >650X for up to 315 cancer-related genes. The results were analyzed for all classes of genomic alterations (GA) including base substitutions, insertions and deletions, select rearrangements, and copy number changes. Tumor mutational burden (TMB) was determined on 1.1 Mbp of sequenced DNA. MSI status was determined by an algorithm based on the sequencing results. TIL were assessed on archived H&E tumor sections and enumerated per guidelines established by the TIL Working Group (Salgado, Ann Oncol 2015) in a subset of MPBC with the highest TMB and compared with low TMB cases.

Results: 165 of mBM cases were MPBC (1.4%) and are included in this study. All patients were female with a median age of 60 (range 24-86). 165 of the MPBC cases (100%) harbored a wide variety of GA involving more than 100 individual genes. The most common GA were identified in TP53 (65%), followed by PIK3CA (37%). No cases of MSI hi status (0/103) and only one case with amplification of 9p21.4 (1/165, 0.6%) were observed. Most MPBC had a low mutational burden, with a median TMB of 2.7 mutations/Mb (range 0-39.6). Only 11/165 tumors (6.7%) were found to have a TMB over 10 mutations/Mb, including 3 cases (1.8%) with TMB >20. Tumor sections were available for TIL review from 9/11 cases with highest TMB, as well as 11 control cases with lowest TMB. TIL were more frequently observed in high versus low TMB MPBC, with median TIL percentage of 40 and 20 (range 10-80 and range 10-60), respectively, although this difference was not statistically significant (Wilcoxon rank-sum test, p=0.15).

Conclusions: Genomic profiling in the largest cohort of MPBC reported to date confirms that MPBC is enriched for TP53 and PIK3CA mutations and many tumors harbor targetable GA. The frequently observed tumoral PD-L1 expression in MPBC is not based on gene amplification as amplification of 9p21.4 is rare. Most tumors had a low mutation burden, and no significant association of TIL with TMB was observed, suggesting additional processes underlying MPBC immunogenicity.
Title: An IL-15 superagonist enhances antibody-dependent cell-mediated cytotoxicity against breast cancer cells regardless of FCGR3A (CD16) genotype and rescues NK cell from TGF-β1-induced immunosuppression

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Body: It has been reported that the Natural killer (NK) cell with FCGR3A (CD16a) V genotype is associated with enhanced clinical response to IgG1 monoclonal Ab (mAb) therapy such as trastuzumab, rituximab and cetuximab (1,2), suggesting a role of antibody-dependent cell-mediated cytotoxicity (ADCC) induced by NK cells. NK cells express three types of polymorphism of CD16; FcγRIIIa-158 VV, VF, FF, which are derived from the genotype of FCGR3A. It is a clinical challenge to improve the outcome in patients with FCGR3A 158FF genotype whose NK cells have lower affinity to mAb and mediate poor ADCC activity. The IL-15 superagonist/IL-15Rα-Fc fusion complex (ALT-803) activates the IL-15 receptor on CD8 T cells and NK cells, inducing their expansion, cytotoxity, and ADCC against B cell lymphoma (3, 4, 5).

Here, we examined the effect of ALT803 on NK cell-mediated ADCC activity by the the anti-HER2 IgG1 mAb trastuzumab in HER2+ cell lines (SKBR3, BT474, MDA-MB-453). In addition, we used the anti-epidermal growth factor receptor(EGFR) IgG1 mAb cetuximab in EGFR positive TNBC cell lines (MDA-MB-231, SUM149, BT549). Finally, we examined the anti-PD-L1 IgG1 mAb avelumab was used for PD-L1 positive breast cancer cell lines (MDA-MB-231, BT549). Trastuzumab, cetuximab, and avelumab all significantly increased NK cell-induced lysis via ADCC. ALT803 significantly further increased both NK induced lysis and ADCC activity in all the cell lines. There was a significant positive correlation for the mean of ADCC lysis induced by NK cells from three FF (21%), three VF (33%), three VV (45%) donors. ALT803 significantly increased the mean of ADCC lysis by NK cells from all donors of each genotype to the same extent. ALT803 increased the expression of NK cell-activating receptors and cytotoxic granules regardless of the genotype of NK cell FCGR3A in terms VV, VF, or FF.

We further examined the potential of ALT803 for NK cell-cytotoxicity suppressed by TGF-β1 which is one of the main barriers to immunity in the tumor microenvironement (TME). NK cells treated with TGF-β1 showed lower expression of activating receptors and cytotoxic granules, culminating in decreased lysis of MDA-MB231. ALT803 inhibited TGF-β1 from down-regulating the expression of NK cell-activating receptors and cytotoxic granules, and from suppressing the cytotoxicity of NK cells to MDA-MB231.

In conclusion, the IL-15 superagonist ALT803 can potentially increase the clinical benefit of ADCC-based mAb therapy for breast cancer patients, regardless of the genotype of FCGR3A. Moreover, ALT803 prevented NK cell-cytotoxicity from TGF-β1-induced suppression, providing a rationale for ALT803 therapy to overcome TME-mediated immunosuppression.

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Title: Class IIa HDAC inhibition promotes an anti-tumor macrophage phenotype that induces breast tumor regression and inhibits metastasis

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Body: While tumor-associated macrophages (TAMs) often have net pro-tumor effects, their embedded location and their untapped potential provide impetus to the discovery of strategies to turn them against tumors. We recently reported that a first in class selective class IIa HDAC inhibitor (TMP195) influenced human monocyte responses to colony stimulating factors CSF-1 and CSF-2 in vitro. Here, we utilize a macrophage-dependent autochthonous mouse model of breast cancer to demonstrate that in vivo TMP195 treatment alters the tumor microenvironment and reduces tumor burden and pulmonary metastases through macrophage modulation. TMP195 induces recruitment and differentiation of highly phagocytic and stimulatory macrophages within tumors. Furthermore, combining TMP195 with chemotherapy regimens or T-cell checkpoint blockade in this model significantly enhances the durability of tumor reduction. These data introduce class IIa HDAC inhibition as a novel means to harness the anti-tumor potential of macrophages to enhance cancer therapy.
Title: Prognostic value of the neutrophil-to-lymphocyte ratio (NLR) and its relation to stromal tumor infiltrating lymphocytes (sTILs) in triple negative breast cancer (TNBC)

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Body: Background: While TNBC remains the most aggressive type of breast cancer (BC), substantial heterogeneity in biology and outcomes exists among TNBC subtypes. Historically, risk stratification of TNBC has been based on anatomic factors such as tumor size, nodal involvement and presence of distant metastases. However, these features alone fail to accurately predict outcomes. Tumor immune infiltration (sTILs) and distribution of immune cell subsets in the peripheral blood (NLR) have emerged as variables reported to be associated with outcomes in TNBC. We sought to evaluate whether NLR and sTILs provided independent prognostic information in TNBC.

Methods: From a cohort of 9,982 women who underwent BC surgery at Mayo Clinic, Rochester, MN between Jan 1985 and Dec 2012, we identified 605 centrally-confirmed TNBC tumors. Patients (pts) with prior BC, bilateral BC, non-invasive disease, stage IV, neoadjuvant therapy, endocrine therapy, or adenoid cystic histology were excluded. For eligible tumors, clinical and pathologic variables were evaluated, including peripheral blood NLR and central assessment of sTILs per the 2014 International TILs Working Group recommendations. We calculated the Pearson correlation coefficient (PCC) between NLR and sTILs and constructed Cox Proportional Hazards Models to evaluate their association with invasive-disease free (IDFS) and overall survival (OS). NLR and sTILs were both analyzed as continuous variables.

Results: Most pts had T1-2 (95%) and N0-1 disease (86%). Median OS follow-up was 10.6yrs. Median IDFS was 12yrs (95%CI 10.2-16.7) and median OS was 18.8yrs (95%CI 15.6-20.8). NLR and sTILs were available in 408 and 599 pts, respectively. The median NLR and sTIL content were 2.29 (0.14-10.50) and 20% (0-90%), respectively. NLR and sTILs were poorly correlated (PCC 0.0237). On univariate analysis (UVA), a higher NLR was associated with worse IDFS (HR 1.13; 95%CI 1.02-1.26, p=0.02) and OS (HR 1.17; 95%CI 1.04-1.31, p=0.01). Each 1% increment in sTILs was associated with improved IDFS (HR 0.99; 95%CI 0.98-0.99, p=0.001) and OS (HR 0.99, 95%CI 0.98-1.00, p<0.001). Among pts with high sTILs (≥20%), a higher NLR remained significantly associated with worse IDFS (HR 1.21; 95%CI 1.05-1.38, p=0.007) and OS (HR 1.25; 95%CI 1.09-1.44, p=0.001). In contrast, among pts with low sTILs (<20%), NLR was not associated with IDFS (HR 1.07; 95%CI 0.89-1.28, p=0.49) or OS (HR 1.07; 95%CI 0.88-1.30, p=0.49). The interaction test between NLR and sTILs did not reach statistical significance. A multivariate analysis (MVA; including age, menopausal status, histologic subtype, grade, tumor size, nodal stage, Ki-67, NLR, sTILs, adjuvant chemotherapy, type of surgery and adjuvant radiation) showed that sTILs remained independently associated with IDFS (HR 0.99, 95%CI 0.97-1.0, p=0.019) and OS (HR 0.99, 95% CI 0.97-1.0, p=0.044), whereas NLR did not.

Conclusions: A lower NLR and a higher sTIL content were each associated with improved IDFS and OS among pts with nonmetastatic TNBC on UVA. However, when evaluated on a MVA, only sTILs remained independently associated with IDFS and OS. Our data suggest that the effect of sTILs on outcomes may not be modified by the NLR.
Title: Improving CAR T cell function by reversing the immunosuppressive tumor environment of breast cancer

Body: Adoptive transfer of T cells redirected to tumor-associated antigens (TAAs) by expression of chimeric antigen receptors (CARs) can produce tumor responses, even in patients with resistant malignancies. To target breast cancer, we generated T cells expressing a CAR directed to the TAA mucin-1 (MUC1). T cells expressing this CAR (86±1.9%, n=5) specifically killed MUC1-expressing cells (MDA-MB-468 – 45.9±7.3%, MCF-7 – 36.8±3.6%) but not MUC1(-) 293T cells (3.7±1.6% specific lysis, 20:1 E:T, n=3). Although these CAR T cells had potent anti-tumor activity against breast cancer cells, when exposed to the Th2-polarizing cytokine IL4 ([which is upregulated in tumor samples (Oncomine, p<0.05)] we observed a dramatic reduction in their cytolytic potential [IL2 - 45.9±7.3% vs IL4 - 11.3±3.7% specific lysis, 20:1 E:T ratio, n=4]. Thus, to protect our CAR.MUC1 T cells from the negative influences of IL4, we generated an inverted cytokine receptor (ICR) in which the IL4 receptor exodomain was fused to the IL7 receptor endodomain (4/7 ICR). Transgenic expression of this molecule in CAR.MUC1 T cells (55±4.8% double positive cells, n=5), restored the cytolytic function of CAR T cells (30.9±8.1% specific lysis, 20:1 E:T, n=3). Next, to determine the long term effects of this modification we co-cultured transgenic T cells with MUC1+ tumor cells and measured tumor and T cells numbers. In the presence of IL4, only double positive (CAR.MUC1-4/7) T cells expanded and eliminated the tumors in vitro and in vivo. However, upon tumor elimination, transgenic T cells rapidly contracted, demonstrating the antigen- and cytokine-dependence of the product. In conclusion, CAR.MUC1-4/7 T cells can effectively target breast cancer cells and retain their cytotoxic function even in the IL4-rich tumor microenvironment.
Title: Lymphoid and myeloid cell characterization of inflammatory breast cancer tumor microenvironment and correlation to pathological complete response

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Background: Inflammatory breast cancer (IBC) is an aggressive form of breast cancer with poor response rates to current chemotherapy regimens. With recent successes of immune targeted therapies in other solid tumors and a growing understanding of how the immune tumor microenvironment (TME) affects non-IBC outcomes, we sought to characterize the immune TME in IBC to identify biomarkers of treatment response and potential targets for drug development.

Methods: Pre-treatment core biopsy samples were identified from the MD Anderson Cancer Center IBC tissue bank from patients with stage III and de novo stage IV (with T4d) disease who received neoadjuvant chemotherapy (NAC) with intent to take to mastectomy. Lymphocytes were characterized by stromal tumor infiltrating lymphocyte (sTIL) quantification, CD8 T cell quantification, and T cell receptor sequencing. PD-L1 expression was assessed using DAKO 22C3 clone on tumor and immune cells. Myeloid cells were characterized using a multiplex immunohistochemistry approach, using CD68 and CD163 for macrophage markers, tryptase for mast cell marker, HLA-DR for class II antigen presentation marker, and cytokeratin as tumor marker. Spatial analyses were performed by determining probabilities of finding cell 1 of interest within 20 uM of cell 2 of interest and computing area under the curve for statistical comparison.

Results: 91 patients with stage III (N=62) or de novo stage IV (n=29) disease were identified. Breast cancer subtype included 25 triple negative, 34 HER2+ and 32 HER2-HR+. 86 patients received a mastectomy, of whom 33 (38.4%) patients experienced a pathologic complete response (pCR). sTIL was higher in stage III tumors (11.9 vs 4.8%, p<0.001) and in those having a pCR (13.8 vs 7.3%, p=0.019). CD8 T cell density (available in 48 cases) similarly was higher in stage III patients (360.3 vs 178.8 counts/mm², p=0.040) and pCR cases (452.3 vs 219.2 counts/mm², p=0.080) but also higher in HER2+ disease (560.9 for HER2+ vs 239.9 counts/mm², p=0.008 for TNBC and 153.6 counts/mm², p=0.005 for HER2-HR+). T cell clonality (available in 32 cases) ranged from 0.004 to 0.242 but showed no correlation to tumor characteristics or response. PD-L1 complete tumor membranous expression was seen in only 1 of 47 cases, whereas PD-L1 positivity on immune cells was seen on 36.2% of cases; neither correlated to response. Myeloid cell assessment (available in 25 cases) showed higher mast cell infiltration in non-pCR cases (63.8 vs 26.8 counts/mm², p=0.008) and spatial analysis (performed on 10 cases) identified that closer proximity of mast cells to CD8 T cells correlates with response (AUC 6.0 vs 2.2, p=0.017), suggesting a possible immunosuppressive mechanism. HLA-DR analysis demonstrated no difference by response as a single stain marker, but co-localization of HLA-DR with cell type shows higher HLA-DR expression on tumor cells in non-responders (14.6 vs 1.6%, p=0.031).

Conclusions: Higher TIL and CD8 T cell density are correlated with improved responses to NAC in IBC. Mast cell infiltration and HLA-DR expression on tumor cells are inversely correlated to response and suggest possible mechanisms of resistance. Mast cells could present potential therapeutic target in IBC.
Title: Prognostic factors for therapeutic personalized peptide vaccines in patients with metastatic recurrent breast cancer

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Body: Background: We have previously reported the prognostic role of humoral antigen spreading response against prostate-related antigen (PRA) for metastatic recurrent breast cancer (mrBC) patients who received personalized peptide vaccine (PPV) therapy (Toh U, SABCS 2015). The prognostic effect was additionally evaluated by the clinical relevant factors including intrinsic subtype, the regimens of combined chemo-hormonal therapies in present study.

Methods: We analyzed serum IgG responses to all of the peptide candidates included PRAs (PSA, PAP and PMSA) after PPV therapy by the Luminex system using peripheral blood samples from 77 vaccinated mrBC patients. The clinical factors and relevant events were statistically evaluated.

Results: After 6 and 12 cycles of PPV therapy, the serum IgG of anti-PSA, anti-PAP, and/or anti-PMSA increased significantly in 31 patients (PRA response group), and the median progression free survival (PFS) and median overall survival (OS) were 8.1 and 14.3 months, but were 5.1 and 10.8 months, respectively, in the remaining 46 patients with no anti-PRA IgG response (PRA non-response Group). The anti-PRA IgG level was marginally correlated with PFS (p=0.059) and OS (p=0.082) between these two groups, which was a significant prognostic factor for PFS (Log-rank: 0.009) in estrogen-positive cancer patients (ER+). The statistical analyses showed that the clinical outcome was in favor of > 60 year-old patients, those with longer PPV therapies (>3 months), and those who received combined standard hormonal therapies or bisphosphonate/anti-RANKL therapy.

Conclusions: This study indicated a clinical significance between the pre-and post- PPV therapy measurement of serum anti-PRA IgG in patients with mrBC, which may be a useful prognostic marker for monitoring peptide vaccine treatment outcomes, particularly for patients ≥ 60 years with ER+ breast cancer. These results also suggest that the immunotherapeutic peptide vaccine could be efficiently combined with hormonal therapy, anti-HER2 therapy, and bisphosphonate/anti-RANKL therapy in mrBC patients.
Impact of neoadjuvant systemic therapy on sTIL in breast cancer

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Body: Background
Multiple studies have shown a correlation between the presence of stromal tumor infiltrating lymphocytes (sTIL) and breast cancer outcomes. Higher sTIL are associated with higher rates of pCR, DFS, and OS. These findings have been primarily observed in TNBC and in HER2+ rather than in HR+, which overall has lower sTIL. Despite this growing body of prognostic evidence, there is little data comparing sTIL at diagnosis to sTIL after neoadjuvant systemic treatment. Understanding the impact of treatment on sTIL may lead to novel techniques to increase host immune response in the tumor microenvironment. The objective of this study was to correlate sTIL in paired samples before and after standard neoadjuvant systemic therapy, and then to identify the direction and magnitude of change in sTIL along with any associated features.

Methods
Institutional pathology database was queried for invasive breast carcinoma with “yp” in staging designation. From July 2013 to September 2016, there were 122 cases identified. When cases were excluded due to unavailable biopsy slides, there were 61 cases remaining. Needle biopsy and final surgical specimens were evaluated using International TIL Working Group 2014 guidelines and reported as a whole number percentage. In specimens with pCR, where there are no applicable guidelines, sTIL were determined by estimating the lymphoplasmacytic infiltrate in the tumor bed. Additional information gathered from the electronic medical record included patient age, clinical TNM stage, breast cancer subtype (HR+, HER2+, TNBC), neoadjuvant systemic therapy regimen (anthracycline, nonanthracycline, Trastuzumab-containing, endocrine), and response to treatment (pCR, residual invasive disease). A negative binomial generalized linear mixed model was used for analysis.

Results
Overall the sTIL declined from biopsy to surgery by about 36% ($\beta = -0.448, S.E. = 0.223, pvalue = 0.045$). However, there were 19/61 patients who had an increase in sTIL with neoadjuvant systemic therapy. For this group, the mean biopsy sTIL was 9.7% while the mean surgery sTIL was 28.7%, significantly higher than the mean biopsy ($p-value < 0.001$). This group was not associated with age, stage, breast cancer subtype, or regimen. We examined our results in the 37/61 patients who did not achieve pCR. In all breast cancer subtypes, this group had an increase from biopsy to surgery in sTIL, with no significant difference between treatment regimens. Increasing age was significantly associated with lower sTIL in both biopsy and surgery specimens ($p-value = 0.033$). For every decade increase in age, sTIL decreased by about 37%.

Discussion
Overall the sTIL declined from biopsy to surgery. In the group of patients with an increase in sTIL, there was no significant correlation with systemic treatment regimen. There was a nonsignificant trend towards increasing sTIL and residual disease at surgery. We hypothesize that the host immune response declined after tumor eradication in pCR and remained active when residual disease was present. Increasing age was significantly associated with lower sTIL. Further work to identify additional factors associated with sTIL is needed to guide efforts to alter the immune response for improved breast cancer outcomes.
Body: Background: African American women have worse prognosis and poor survival rates due to lack of Androgen Receptor expression. These subtypes, the Quadruple Negative Breast Cancers (QNBC), currently have no treatments. Previously, we found that AA TNBC have 28% more chance of being QNBC and have 25% higher rate of progression vs the Caucasian Americans (CA). Also, these QNBC patients have a unique immune and basal signature. Here, we explore individual genes and their correlation to race and immune therapies.

Methods: We analyzed AR mRNA expression in 925 tumors from CA or AA women from The Cancer Genome Atlas and independently validated AR protein levels by immunohistochemistry in 197 primary tumors. Samples were dichotomized as AR positive or negative using mean gene expression cutoff. Gene expression comparisons among groups were determined using standard t-tests, ANOVA, and odds ratios with a significance threshold alpha of 0.05. Therapy targets were selected from the list of significantly differentiated genes. Boxplots and heatmaps were made using R packages. Cumulative Incidence plots for time to progression were constructed using XLStats and Grays test for significance.

Results: QNBC CA have higher expression of PDCD4 (p <.001), CD46 (p <.001), CD3EAP (p =.04) and CD84 (p =.07) compared to QNBC AA. CD47 (p =.63) and PD1 (p =.96) express no significant differences in race. QNBC AA show higher expression of NFKB (p <.001), CCL2 (p <.001), and IL12 (p <.001) compared to QNBC CAs. Interestingly, QNBCs with high expressions of CD47 (p =.04) have poor prognosis. Thus, QNBC patients with low expression of CD46 (p =.025), PDCD4 (p < .001), and CD3EAP (p < .001) have poor prognosis and overall survival.

Conclusions: Current immune targeted therapies like CD47 and PD1 looks favorable for treatment in some QNBC patients. Race appears to effect other major therapy target gene expressions in the QNBC immune basal signature as well. This may imply that QNBC AA and CA have different immune response which may explain racial progression differences. Assessment of immune markers may contribute to more accurate prognosis.
Title: VISTA expression on tumor-infiltrating lymphocytes in breast cancer: Clinical correlates and association with PD-1

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Body: Background: The V-domain containing Ig Suppressor of T Cell Activation (VISTA) is a recently discovered immune checkpoint receptor with homology to PD-1. VISTA expression on tumor-infiltrating lymphocytes (TILs) blocks their proliferation and effector functions. Recent pre-clinical data in cancer mouse models treated with anti-VISTA and anti-PD-L1 combinations showed promising results and non-redundant functions of VISTA and PD-L1 blockade. However, the expression and prognostic value of VISTA+ TILs in primary breast tumors has not been investigated in detail. Here we assess expression, prognostic value, and associations of VISTA+TILs with other immune checkpoint markers (PD-1/PD-L1, LAG-3, and IDO-1) and with H&E TIL counts.

Methods: A tissue microarray consisting of breast carcinoma primary excision specimens (n=330) from the University of British Columbia hospital, linked to detailed clinicopathological data and outcomes, was used in this study. Patients from this cohort did not receive neoadjuvant treatment. A VISTA antibody (Clone D1L2G) was applied on a 4µm section of the tissue microarray by immunohistochemistry using a Ventana automated stainer. VISTA+TILs in direct contact with tumor nest were scored and reported as absolute count per 0.6mm core. Positive cases were defined as cases with VISTA+TILs ≥ 1. All descriptive and survival analyses were conducted using SPSS software.

Results: VISTA+TILs were present in 30% of cases and were significantly (p<0.05) associated with younger age (<50 years old), larger tumors (>2cm), hormone receptor negativity (ER/PR) and high Ki67 proliferation index (≥ 13.25%). Almost half (48%) of basal-like breast cancers were positive for VISTA expressing TILs. No significant prognostic associations were observed in this cohort. Among the immune checkpoint receptors analyzed, VISTA+TILs were highly associated with PD-1+ TILs: 79% of cases positive for PD-1+TILs were also infiltrated by VISTA+TILs. Interestingly, we found that VISTA+TILs and PD-1+TILs were enriched in cases with otherwise low levels (<10%) of H&E TILs.

Conclusions: Our study identifies the presence of VISTA+TILs in breast cancer patients and its strong association with PD-1+TILs. These results suggest that VISTA blockade could be a good candidate for combination therapy with other immune checkpoint inhibitors, a concept being tested in early phase clinical trials. Validation of these findings in a larger independent cohort powered for multivariate analysis is currently ongoing.
Title: The neo-epitope landscape of breast cancer: Implications for immunotherapy

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Body: Introduction: Immune checkpoint blockade is an effective immunotherapy for multiple cancers, including a subset of TNBCs. The clinical response to checkpoint blockade correlates with high tumor mutational burden. Cancer-induced mutations are predicted to generate new HLA-binding epitopes (neo-epitopes), which are potential targets of T cells. To determine the neo-epitope load in breast cancer, we developed a rapid bioinformatics pipeline and filtering strategy, EpitopeHunter, to identify and prioritize clinically relevant neo-epitopes from the landscape of somatic mutations. Here, we determined the specificity and frequency of neo-epitopes from the TCGA dataset of invasive breast cancers.

Methods: We analyzed 842 tumor and normal breast cancer exome sequencing from the TCGA dataset. Subgroup analysis was performed based on the IHC status of ER, PR and HER2: (i) TNBC (n=98); (ii) ER and/or PR(+), Her2(-) (n=604); and (iii) ER and PR(+/-), Her-2(+) (n=140). Cancer specific mutations were called uniformly for the aligned tumor and normal pairs using VarScan2. For each annotated non-synonymous mutation, we generated all possible neo-epitopes (up to 11-mers) including the mutant amino acid. HLA typing of A, B and C MHC class I genes of each patient was determined using the POLYSOLVER algorithm. We selected high affinity binding epitopes using the IEDB prediction algorithm based on the known epitopes to identify likely binding of each neo-epitope within the suite of patient-specific HLA alleles (IEDB score < 500). We then filtered the epitopes based on tumor transcript abundance using RNAseq expression for 837/842 patients. An expression value of FPKM > 2 was used as a cut-off for the expressed neo-epitopes.

Results: Total mutational burden was highest for TNBC (median=53, range: 2-1162); followed by ER and PR(+/-), Her-2(+) (median=40, range: 6-4983); and lowest for ER and/or PR (+), Her-2(-) (median=30, range: 0-5515). About 15% of the nonsynonymous mutations led to the generation of neo-epitopes. The neo-epitope load (high affinity neo-epitopes with IEDB score <500) is highly correlated with the mutational burden (R^2= 0.8-0.9). Number of patients with at least one neo-epitope expressed in each category was: TNBC (n= 79/97, 81%); ER and/or PR(+), Her2(-) (n=478/600, 79%); and ER and PR(+/-), Her-2(+) (n=115/140, 82%). Overall, 672/837 patients have at least one predicted high affinity neo-epitope expressed, with an average of 14.7 (1-525) neo-epitopes expressed per patient.

Conclusions: We have developed a rapid bioinformatics pipeline, EpitopeHunter, for the identification of neo-epitopes from tumor sequencing data. Eighty percent of breast cancer patients have at least one expressed neo-epitope, which may serve as candidates for targeted immunotherapy.
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Title: Expression of the tumor associated carbohydrate antigen Tn and immune effectors in invasive breast cancer

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Body: Background:
Glycosylation is a post-translational modification generating complex carbohydrate O-glycans. Tn antigen, a N-acetyl-galactosamine-α-O-Ser/Thr residue that is the core glycosylated component of complex mucin-type O-glycans, is expressed in breast cancers. Tn has been associated with invasion, survival and decreased T-cell mediated immune response in several types of cancer including breast cancer. There is therefore a potential rationale to combine Tn vaccine with checkpoint blockers such as PD-1/PDL-1 inhibitors. Little is known regarding the link between the respective expression of Tn antigen, PDL-1, and TILs, in breast cancer. Our study aimed at characterizing Tn expression in a series of invasive breast cancers, and its correlation with immune response.

Methods
Tn expression was analyzed by immunohistochemistry performed on a tissue microarray of 1864 invasive breast cancer samples (1850 patients for statistical analyses), consisting of 189 (10%) TNBC, 1601 (86%) ER and/or PR positive à checker tumors and 191 (10%) HER-2 positive tumors treated at our institution between 2005 and 2013. Among these cases, 50 luminal B tumors and 50 TNBC were selected for full section and immune analysis, i.e. to study the pattern of Tn expression across whole tumor section and the link between Tn expression and the level of CD3+ and CD8+ infiltrates, and PDL-1 expression. The level of Tn expression was assessed using H-score, combining the percentage of stained cells with staining intensity.

Results:
In the 100 initial samples, Tn expression was observed in 95%, with a H-Score>10 in 85% of the cases, and with homogeneous staining across the whole tumor section in 86% of the cases. Tn expression was increased in Luminal B subtype as compared to TNBC, with a mean H-Score of 100.9 vs 55, respectively (p <0.0001). The CD3+ infiltrate was more important in TNBC as compared to luminal B tumors (mean stromal CD3+: 34.3% vs 21.2%, p=0.0052). No significant difference was found for the CD8+ infiltrate. PDL-1 expression in stromal cells (≥1%) was observed in 43% of the cases, and increased in TBNC as compared to Luminal B (mean of 7.3% vs 2.2% of cells respectively, p=0.0252). In both tumor subtypes, we observed a positive correlation between the CD3/CD8 infiltrates and PDL-1 expression in stroma cells (p<0.0001), but not with Tn expression. In the TMA cohort, Tn expression was observed in 1723 (92%) tumors with a mean H-Score of 82.75. Similarly, to the results obtained in full section, Tn expression was less intense in TNBC as compared to other subtypes (mean H-Score of 62 vs 85, p<0.001). Tn expression was lower in histological grade I as compared to grade II/III tumors (64,69 for grade I, 88,84 for grade II and 88.99 for grade III, p<0.0001), and significantly higher in HER2 positive tumors versus others (mean H score 62,06 for HER2-/RE-, 119,05 for HER2+ and 80,25 for HER2-/RE+, p<0.0001).

Conclusion:
Our study shows a lower level of expression of Tn antigen in TNBC as compared to luminal tumors. We could not identify any correlation between Tn antigen expression and immune status of the tumor as defined by CD3+, CD8+ and PDL-1+ infiltrate in tumor stroma. The role of Tn antigen in the HER2-positive subgroup requires further investigation.
Title: PD-1 expression and its correlation with tumour infiltrating lymphocytes and Ki67 in patients with breast cancer

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Body: Introduction: Immunotherapy targeting the programmed death-1 (PD-1) checkpoint has resulted in good outcomes in patients with melanoma, non-small cell lung cancer and head and neck cancer. Recent data have showed that patients with ER/PR negative breast cancer might benefit from immunotherapy as well. We investigated PD-1 expression on tumour infiltrating lymphocytes (TILs), the presence of TILs and Ki67. Furthermore, the association of PD-1, TILs and Ki67 with time to metastasis was statistically determined.

Methods: Resection specimens of 284 patients with an ER/PR negative invasive ductal adenocarcinoma of at least 2 cm were analysed. Haematoxylin-eosin staining was performed to determine the percentage of TILs. Subsequently these percentages were divided into three groups: less than 10%, 10%-30% and more than 30% which matches with respectively 30%, 50% and 20% of the patients. PD-1 expression on the TILs of 116 patients and Ki67 expression of 185 patients were assessed using immunohistochemistry.

Results: An increase in the fraction of TILs was significantly associated with a lower risk of metastasis (hazard ratio [HR] = 0.7, 95% CI = 0.6-0.9). PD-1 expression on TILs was observed in 97% of the patients. Three patients had no expression of PD-1, this lack of PD-1 expression significantly increased the risk of metastasis (HR = 5.541, 95% CI = 1.2-25.0). No association could be noted between PD-1 expression and the amount of TILs or Ki67 (p-values of respectively 0.272 and 0.637). Similarly, no association could be noted between Ki67 and the amount of TILs (p-value = 0.1954).

Conclusion: PD-1 was expressed on TILs in patients with breast cancer. Surprisingly, no correlation was found between PD-1 expression and TILs or Ki67. However, an increased amount of TILs was significantly associated with a lower risk of metastasis.
Title: A mathematical model for predicting immune response in a trastuzumab treated HER2+ breast cancer animal model: Preliminary efforts

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Body: Introduction: Trastuzumab is a targeted antibody to the human epidermal growth factor receptor 2 (HER2) that induces cell cycle arrest and is used in the treatment of HER2+ positive breast cancer. We have recently presented in vivo evidence in a murine model that trastuzumab also improves vascular delivery of subsequent cytotoxic therapies. The mechanism by which trastuzumab and the immune system interact to regulate tumor-associated angiogenesis is not well characterized. Therefore, we offer a preliminary report on a mathematical framework to systematically investigate the potential interactions among the immune response, tumor cells, vasculature, and other environmental factors based on experimental data for the BT474 murine model of HER2+ breast cancer.

Experimental: BT474 breast cancer cells were implanted subcutaneously into athymic nude mice. After tumors reached 250 mm^3, mice were treated with trastuzumab or saline, tumor volumes were recorded, and tumors were extracted at various times over seven days. Immunohistochemistry for treated and control tumors were evaluated for 0, 1, 3, 4, and 7 days post trastuzumab treatment. Histology data includes: percent hypoxia (pimonidazole), percent necrosis, and vascular maturation index (VMI, ratio of alpha-smooth muscle actin to total vessel counts as stained with CD31). Ongoing studies are quantifying immune cell infiltration through immunofluorescent imaging of F4/80 and CD11c expression.

Modeling: We developed a system of five coupled, ordinary differential equations that accounts for the temporal variation in tumor growth, vasculature, hypoxia, necrosis, and immune response. The general immune response component corresponds to the mouse's pro-inflammatory responses, as T cell driven responses are absent in this murine model. Uncertainty analysis was performed to verify plausible overlap between the model's predictions and the experimental data—where local and global samplings of all parameters were used to generate potential model results to be compared to the data for both control and treated mouse sets. Sensitivity analysis was performed using Sobol' Indices to determine the driving parameters of the system to identify target parameters for experimental estimation. The model parameters were calibrated using mean and standard deviations for the available data (tumor volume, VMI, percentage of hypoxia, and percentage of necrosis) for each experimental time point to predict the differences of the immune component between the treated and control tumors.

Results and Discussion: The model is well behaved and can be calibrated with the available data. The model predicts distinct differences for the immune response between the control and treated groups—showing increasing versus decreasing immune component values over time for treated versus control results, respectively. Preliminary results from the immunofluorescent imaging data support the immunological predictions of the model; in particular, the amount of immune infiltration (i.e., more immune cells) in necrotic areas is greater in the treated than the untreated tumors (p<0.025).

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Title: The numbers of Foxp3 positive cells in simultaneous bilateral breast cancer

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Body: Background
Evasion of the immune system is one of the hallmarks of malignant tumors, and recently blocking of such evasion has been used in clinical practice for the treatment of some types of cancers. Recruitment of regulatory T cells (Tregs), which express the specific marker Foxp3, is an established mechanism of escaping from the immune system. In breast cancer, it has been reported that the number of Tregs differs widely among breast cancer subtypes, and that patients who have many Tregs around the tumor tend to have a poor prognosis. However, the factors that are important for the recruitment of Tregs are not well known. Thus, factors that depend on the host (e.g., age or comorbidity), on the tumor (e.g., subtype, grade, or stage), or on measurement error might be the cause of the observed differences in the number of Tregs. In this study, we investigated the numbers of Tregs in simultaneous bilateral breast cancer patients in order to determine the factors that influence the recruitment of Tregs, while excluding differences in individuals as much as possible.

Material and methods
Patients who had breast cancer in both breasts and who underwent simultaneous surgery between January 2005 and September 2015 at two institutions were enrolled in this study. Patients who underwent primary systemic therapy who were diagnosed with ductal carcinoma in situ, or who were stage IV were excluded. The average numbers of Foxp3-positive (Foxp3+cells) were determined from scores of five high-power fields (HPFs). The association between the difference in Foxp3+ cell number between each breast in a single individual and clinicopathological features was examined.

Results
Seventy patients were included in this study. Their ages ranged from 39-85 (median 54) years old. Ninety five percent of the tumors were invasive ductal carcinoma non-special type. Eighty eight (62.9%), 43 (30.7%), and 9 (6.4%) of the tumors were T1, T2, and ≥T3, respectively, and 102 (72.9%) of the tumors were node-negative. Regarding nuclear grade (NG), 104 (74.3%), 21 (22.1%), and 7 (5%) of the tumors were NG1, 2, and 3, respectively. As for subtype, 124 (88.6%), 9 (6.4%), and 7 (5%) were ER-positive and HER2-negative(ER+/HER2-), ER-positive or negative and HER2-negative(ER±/HER2+), and ER-negative and HER2-negative(ER-/HER2-), respectively. The numbers of Foxp3+ cells ranged from 0 to 39.8 (median 3.3)/HPF, and difference in Foxp3+ cell number between each breast in a single individual ranged from 0 to 34 (median 3.9)/HPF.

Differences in tumor size and node status in individuals did not impact on the number of Foxp3+ cells. However, the number of Foxp3+ cells in tumors that were NG3 (P=0.00098) or ER±/HER2+ or ER-/HER2- type (P=0.00586) were statistically significantly increased compared with tumors that were NG1/2 or ER+/HER2- type in the same host.

Furthermore, the difference of Foxp3+ cells between each tumor in a single individual were quite small regarding tumor size and node status in 53 patients who had similar NG and subtype tumors in both breasts.

Conclusions
The number of Foxp3+ cells showed no relationship with tumor size, or lymph node status in simultaneous bilateral breast cancer patients. High NG, ER±/HER2+ or ER-/HER2- type of the tumor were involved with enhancement of the recruitment of Tregs.
2017 San Antonio Breast Cancer Symposium

Publication Number: P3-05-18

Title: The complex landscape of breast cancer immune evasion and its implications to personalized and combined immunotherapy

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Body: Cancer immunotherapy activates the patient’s immune system to recognize and kill cancer cells. The efficacy of immunotherapy in human breast cancer (BRCA) is hindered by the poor understanding of the immune system’s anti-tumor response and tumor evasive mechanisms, and the lack of optimized treatment regimens for each BRCA patient. There are 6 known evasion mechanisms (M) by which tumors can escape immune surveillance. M1: immunosuppression via interleukin 10 (IL-10) and tumor growth factor beta (TGF-β); M2: induction of tolerance by cytotoxic T-lymphocyte associated protein 4 (CTLA-4), programmed cell death protein 1 (PD-1) and its ligands (PD-L1/PD-L2), and interferon gamma (IFN-γ); M3: apoptosis resistance via the expression of anti-apoptotic molecules, namely baculoviral IAP repeat-containing protein 3 (BIRC3), TNF receptor associated factor 1 (TRAF1), and TNF alpha induced protein 3 (TNFAIP3); M4: counterattack via decoy death receptors; M5: impaired antigen presentation by decreasing beta 2 microglobulin (B2M) and human leukocyte antigens A and B expression (HLA-A and HLA-B); and M6: ignorance caused by the absence of a danger signal or an antigen. Yet, which mechanisms or combination of mechanisms BRCA tumors utilize is unknown. Moreover, the currently used biomarkers for the choice of immunotherapy are not ideal, as for example, not all PD-L1⁺ patients respond to anti-PD-L1 treatment, and certain PD-L1⁻ patients respond. Thus, there is a need for more precise biomarkers. Using the sequential biclustering method on the RNA-sequenced data of BRCA from The Cancer Genome Atlas (TCGA), eighty-one percent of BRCA patients were grouped into 7 different clusters (CL) of immune evasion subtypes. We found that the majority of breast cancer tumors utilize multiple immune evasion mechanisms. Tumors may use from 1 up to 4 combinatorial mechanisms simultaneously. We also identified classifier genes that would help sort patients based on their evasion mechanisms, and guide the choice of immunotherapy. This is especially important since the immune evasion molecules may not be the ideal biomarkers for the best choice of immunotherapy. Moreover, we showed that triple negative breast cancer (TNBC) patients were significantly associated with CL2 (M5).
Title: Regorafenib induces immunogenic cell death via p-stat3 inhibition in triple negative breast cancer cells

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Body: Background
Metastatic triple-negative breast cancer (TNBC), despite being chemo-sensitive, remains poor prognostic. Rising data have suggested some anti-cancer agents exert immunostimulatory activities through inducing immunogenic cell death (ICD). ICD can be detected by presence of damage-associated molecular patterns (DAMPs) such as cell surface exposure of calreticulin (CRT), secretion of ATP, and increase in high-mobility group box protein B1 (HMGB1) release from dying tumor cells. Signal transducer and activator of transcription 3 (STAT3) activity is activated in TNBC and regorafenib has been shown to suppress p-STAT3 signal. We tested whether regorafenib induces ICD in TNBC.

Methods
Mice TNBC cell line 4T1 cells were treated with regorafenib and cell survival was examined by MTT assay and flow cytometric analysis. DAMPs were examined by western blot analysis, immunofluorescence microscopy and luminescent assay. The correlation between regorafenib-mediated ICD and STAT3 inhibition were validated in ectopic STAT3 transfected 4T1 cells. Moreover, we investigated the combination strategies of regorafenib with immune checkpoint blockade in syngeneic 4T1 tumor bearing mice (TNBC animal model) with treatment of regorafenib or mPD1 treatment alone/ combination of mPD1 and regorafenib.

Results
The results demonstrated that regorafenib reduced cell survival and induced cell apoptosis in a dose-dependent pattern in 4T1 cell line. Regorafenib induced ICD, as evidenced by it triggered the release of HMGB1 and ATP, as well as the exposure of CRT on the cell surface. Moreover, regorafenib-induced ICD was attenuated by ectopic expression of STAT3 (thus increased phosphorylation of STAT3) in STAT3-overexpressed 4T1 cells. Last but not the least, we observed 4T1 tumor bearing mice with treatment of regorafenib alone, or anti-PD1 monoclonal antibody (mAb) alone, or in combination of regorafenib and anti–PD1 mAb resulted in reduced size of primary tumors, increased survival and fewer lung metastases. Flow cytometric analysis revealed regorafenib treatment increased activated CD8+ T cells, increased antigen-presenting dendritic cells, and suppressed regulatory T cells (Tregs) in mice spleen.

Conclusions
Regorafenib induced ICD in 4T1 cells via the inhibition of p-STAT3. Regorafenib also promoted CD8+ T cells activation and antigen presenting ability of dendritic cells, and suppressed Tregs. Our study demonstrated regorafenib as an ICD-inducer and immunomodulator and its potential combination with immune checkpoint blockade in TNBC.
Title: A study on amount, localization and immune phenotype of tumor-infiltrating lymphocytes in different subtypes of breast cancer

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Body: Objective: The aim of the study was to investigate the localization, amount and type of lymphocytic infiltrate, found in different molecular subtypes of breast cancer (BC).

Methods: Retrospectively, 100 cases of invasive BC were analyzed and immunohistochemically (IHC) stratified in four subtypes (Luminal A and Luminal B-like, HER2-positive, and triple negative (TN). The percentage of stromal areas occupied by tumor-infiltrating lymphocytes (TILs) was assessed for H&E slides. The samples were IHC-stained for CD3, CD4, CD8, CD20 and FoxP3. The immunophenotyped lymphocytes - intratumoural and stromal, were separately counted, semi-quantitatively graded and further analyzed.

Results: A total of 10% of all tumors were lymphocyte-predominant BC. Intratumoral and stromal TILs were predominantly CD3+T-lymphocytes. High counts of all subtypes TILs - intratumoral and stromal, were most common for TNBC and HER2-positive BC.

In TNBC, the intratumoral CD3+TILs are significantly related to CD8+ (p=0.002) and FoxP3+ phenotype (p=0.010). In HER2 BC, the intratumoral and stromal CD3+ TILs were significantly related to FoxP3+ phenotype (p = 0.035 and p= 0.011, respectively).

Conclusion: CD3+T-cell mediated immunity, especially the one related to CD8+ and FoxP3+ lymphocytes was the leading one in antitumor response in BC, and high count of intratumoral and stromal lymphocytes predominated in TN and HER2-positive BC.

Key words: Breast cancer, tumor-infiltrating lymphocytes, CD3, CD4, CD8, CD20, FoxP3.
Title: An integrative approach to the discovery of triple-negative breast cancer markers derived from extracellular vesicles

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Body: Triple-negative breast cancer (TNBC) is an aggressive subtype accounting for nearly 20% of all breast cancer (BC) cases with disproportionally poor prognosis. Treatment options are limited by the lack of expression of common BC receptors targeted by FDA-approved small molecule inhibitors, resulting in an unmet need for efficient and cost effective treatments. Immunotherapies are emerging as a powerful alternative to standard chemo- and radiation therapy. Our objectives are to generate vaccines targeting TNBC and to identify circulating biomarkers capable of monitoring their therapeutic efficacy in a human transgenic mouse model (C57BL/6) compatible with EO771.LMB, a murine cell line of TNBC with a reported basal-like phenotype.

Our current study characterized the cancer-related proteomic and metabolic signatures derived from extracellular vesicles isolated from EO771.LMB. It has been shown that extracellular vesicles (EV) shed by tumors carry complex cargos comprised of proteins, metabolites and nucleic acids and may exhibit cancer-specific signatures with a potential as prognostic markers or predictors of therapy response. EVs released by TNBC tumors into circulation might provide non-invasive and highly actionable insight into the tumor biology of this aggressive cancer.

To further our understanding of cancer-specific EV proteomes, we used mass-spectrometry based proteomics and identified a total of 2265 unique proteins from EVs isolated from conditioned media from EO771.LMB. Our survey across the EV proteome, the expression proteome (5096) and phosphoproteome (2728) identified 6461 unique proteins overall. Within EV sub-fractions, microvesicles and apoptotic bodies were characterized by proteomic cargos different from exosomes. Over 90% of proteins identified in EVs shed by cultured EO771.LMB mapped with proteins curated in ExoCarta. Of interest, exploration of the EV proteome identified 47 GPI-anchored surface proteins. These candidate membrane proteins included Glypican-1 and CD109, previously reported as highly specific to cancer, as well as potential TNBC-specific markers. Our multipronged strategy for deep proteomic profiling of EO771.LMB enhanced identification by ~25% compared to global whole cell proteomics alone. We also identified ~3150 metabolites corresponding to lysophosphatidyl choline (35%), fatty-acids, -esters, -amides, -alcohols (30%), glycerophospholipids and sphingolipids (16%), sugar (1%), amino acids and biogenic amines (4%), organic acids and derivatives, cyclic alcohols, aromatic compounds and steroids (13%). Of these, 1450 metabolites were shared between cell line and EVs. A proteogenomic approach expanded identification to onco-proteoforms and tumor-specific peptides not represented in canonical databases. Custom, cell-specific protein databases derived from whole transcriptome sequencing of EO771.LMB elucidated novel protein variants and mutations. Pan-cancer markers vs. TNBC-specific markers will be verified in other murine cell lines, across multiple cancer types and fibroblast controls. Optimal membrane markers for downstream immune-affinity purification from plasma will be proposed, and their ability to diagnose disease or monitor tumor load during treatment will be discussed.
Anti-uPAR antibody drug conjugates for targeted therapy of triple-negative breast cancer

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Body: Background: Triple negative Breast Cancer (TNBC) is a highly aggressive BC subtype, with an increased likelihood of distant recurrence and of death compared with other types of BC. Patients diagnosed with TNBC lack the estrogen and progesterone receptors, the human epidermal growth factor 2 receptor and do not respond well to current therapies. One hallmark of TNBC is increased uPAR expression. As uPAR was observed in BC and its metastasis but not in normal tissue, anti-uPAR Abs can serve as a targeted therapy for TNBC. The Craik lab used recombinant phage display technology to identify anti-human-uPAR Abs. Two of those human antibodies, 2G10 and 3C6, were tested in in vitro and in vivo TNBC models and demonstrated therapeutic potential. In the mouse model of TNBC the “naked” Abs targeting two distinct subdomains of the receptor slowed or blocked tumor growth. Moving forward in validating the antibodies as a therapeutic for aggressive breast cancer, we have chosen to develop them as a ‘weaponized antibody’ therapy in the form of antibody-drug conjugates (ADCs). Excellent proof of concept for the therapeutic potential of anti uPAR ADC was achieved with anti uPAR (2G10) conjugated to the therapeutic radioisotope 177Lu. With the current limitations of radiotherapy we conjugated 2G10 and 3C6 to make ADCs with a known cytotoxic agent that is available for the treatment of cancer.

Hypothesis: We hypothesize that human anti uPAR mAbs, when coupled to a cytotoxic drug will demonstrate cancer-selective cell killing in mouse xenograft models of TNBC, and are safe with a large therapeutic window, thereby validating uPAR as a therapeutic target for TNBC and anti-uPAR antibodies as a potential therapeutic.

Study design: We tested the ability of the ADCs to target the urokinase receptor that is over-expressed in breast cancer, for therapy. Six- SMART TAG (CATALENT) based anti uPAR (2G10 and 3C6) ADCs have been assembled and characterized. The 6 ADCs with cleavable and non-cleavable linkers, 3 different toxins (alkylation agent and antimotic agents) and drug antibody ratio (DAR) of~2 were produced in reagent quantities. The ADCs were tested in vitro in head-to-head comparisons between the ADC form and unconjugated mAb for their cell killing ability using cell culture assays. Further characterization was performed in vivo TNBC models.

Results: The anti uPAR ADCs were able to recognize uPAR in TNBC cell lines. The ADC 3C6 AzaHIPS-4AP-MMAE exhibited the greatest therapeutic effect in vitro in TNBC cell lines and showed significant slowing or blocking tumor growth in a mouse model of TNBC.

Conclusions: Our results indicate the therapeutic potential of anti-uPAR ADC for TNBC.

Acknowledgments: This work was supported by the Target Validation Initiative grant from the UCSF Helen Diller Family Comprehensive Cancer Center and a grant from Susan G. Komen (PDF15330246) made possible through fundings from American Airlines.
**Body: Introduction:** Secreted frizzled related protein-2 (SFRP2) is overexpressed in breast tumor endothelium and epithelium, and stimulates angiogenesis via the calcineurin/NFAT pathway. We hypothesized that a humanized SFRP2 monoclonal antibody (mAb) would inhibit tumor growth.

**Methods:** Humanization of SFRP2 mAb. V region genes encoding the murine SFRP2 mAb were cloned and used to construct chimeric antibodies comprising the murine V regions combined with human IgG1 heavy chain constant regions and κ light chain constant regions, were expressed in HEK293 cells, purified and tested for binding to SFRP2 in a competition ELISA assay. The antibodies were purified from cell culture. Immunogenicity testing of hSFRP2 mAb. The lead fully humanized and chimeric anti-SFRP2 antibodies were tested against a cohort of 22 healthy donors using EpiScreen™ time course T cell assay in order to determine the relative risk of immunogenicity. Endothelial tube formation assay: Cells were plated in Matrigel alone, with SFRP2 30nM, IgG1 control + SFRP2 30nM, or SFRP2 30nM + a dose range of hSFRP2 mAb from 0.1-10 uM. Tubes were quantified after 4 hours. Determination of maximum allowable dose of hSFRP2 mAb in mice. 1 x 10^6 SVR angiosarcoma cells were injected s.c. into nude mice. Mice were treated with 1, 2, 4, 10 and 20 mg/kg iv of hSFRP2 mAb q 3 days for 3 weeks. The livers and kidneys were resected, and paraffin embedded sections were stained with hematoxylin and eosin. Next we injected 5 x 10^6 hS578t triple negative breast cancer into the mammary fat pad on female nude mice, and treatment was started when tumors reached apx 100 mm^3 after 37 days. Control mice received IgG1 control (4 mg/kg) iv q3 days, and treated mice received hSFRP2 mAb 4 mg/kg iv q3 days for 5 weeks.

**Results:** Immunogenicity testing. The hSFRP2 mAb induced no positive responses using SI ≥ 2.0, p < 0.05 threshold in any of the donors in the proliferation assay, whereas the chimeric anti-SFRP2 antibody induced positive T cell proliferation responses in 23% of donors. Endothelial tube formation in vitro. The hSFRP2 mAb inhibited SFRP2 induced endothelial tube formation in a dose dependent manner with an IC50 of 2.7 µM. Inhibition of tumor growth in vivo. Angiosarcoma: There was no weight loss or lethargy in any of the mice after 3 weeks of treatment at any of the doses, including the 20 mg/kg dose. Control and group 20 mg/kg livers and kidneys showed no abnormalities detected in any of these organs. We tested efficacy of the hSFRP2 mAb at a dose of 4 mg/kg, using 10 mice per group. This shows a statistically significant reduction in tumor volume by 40% in the hSFRP2 mAb compared to control (p=0.04). For hs578t breast tumors here was a 57% reduction in tumor volume in hSFRP2 mAb compared to control (793 mm^3 ±72 vs. 341 mm^3 ± 25, n=10, p<0.0001).

**Conclusion:** We have generated a novel humanized SFRP2 mAb that lacks immunogenicity and inhibits triple negative breast cancer and angiosarcoma in vivo as a single agent without toxicity.
Title: Tackling bone metastatic breast cancer growth with novel bone-seeking matrix metalloproteinase-2 inhibitors

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Body: Background. Despite medical advances, currently there is no treatment for breast to bone metastasis. The progression of bone metastatic breast cancer is critically dependent on interactions with the surrounding microenvironment. Therefore, identifying the underpinning molecular mechanisms is vital for the development of new therapies.

Rationale. Gene expression analysis and validation in human and murine specimens of bone metastases revealed matrix metalloproteinases, such as MMP-2, are highly expressed in the bone metastatic microenvironment. Genetic ablation of MMP-2 demonstrated the importance of this MMP in driving the growth of the osteolytic bone metastatic breast cancer by regulating the bioavailability of transforming growth factor β (TGFβ). These data support the rationale for the development of a highly specific MMP-2 inhibitor for the eradication of active bone metastatic breast cancer.

Methods. We utilized a novel chemical approach to synthesize bone seeking MMP inhibitors (BMMPIs) on a bisphosphonic backbone, with specificity for MMP-2 in the nanomolar range (IC₅₀=140 nM). In vitro, we tested the effect of BMMPIs at varying doses (1nM-100µM) on the viability of the major cellular components of the cancer-bone microenvironment, namely breast cancer cells (PyMT, 4T1), osteoblasts (MC3T3) and osteoclasts (primary monocytes and RAW 264.7). In vivo, mice were intratibially inoculated with either luciferase expressing 4T1 or PyMT (1x10⁵) cells. Mice (n=10/group) then received vehicle, zoledronate (1 mg/kg) or BMMPIs (1 mg/kg). Tumor growth was determined via luminescence quantitation. Cancer induced bone disease was measured ex vivo by µCT, Xray and histomorphometry. MMP activity in vivo and ex vivo was determined via specific activatable MMP probes. Pharmacokinetic and pharmacodynamic studies were performed. Plasma and bone marrow supernatants were collected from PyMT-R221A tumor bearing mice treated with ML115 (5mg/Kg) at 0.25, 0.5, 1, 2, 4, 8, 24 hours and three weeks (n=3 mice/time point). Currently, we are investigating the BMMPIs ability to impact the metastatic process through an in vivo model of intracardiac inoculation.

Results. BMMPIs significantly impacted the viability of breast cancer cells and osteoclasts in vitro (p<0.05) compared to control. In vivo, BMMPIs significantly reduced the growth of bone metastatic breast cancer compared to control and the standard of care bisphosphonate, zoledronate. MMP activity was also lower in the BMMPI treated groups (using tumor burden to normalize values). µCT/Xray/Histomorphometry analysis also illustrated the significant beneficial effects of the BMMPIs in reducing the size of osteolytic lesions (up to 80% by µCT; p<0.05). ML115 is rapidly cleared from the plasma and accumulates selectively in the bone marrow microenvironment over time.

Conclusions. MMP-2 specific BMMPIs prevent bone metastatic breast cancer growth by impacting cancer cell viability and cancer induced osteolysis. Given that bisphosphonates are well tolerated in the clinical setting, we predict that BMMPIs could be translated to the clinical setting for the treatment and eradication of bone metastatic breast cancer.
Title: Dual p38/NLK kinase inhibitor as potential novel therapeutic agent for tamoxifen-resistant luminal breast cancer

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Body: Background: Tamoxifen is the most commonly used endocrine agent for estrogen receptor (ER) positive breast cancer (also known as luminal breast cancer). However, approximately half of the patients develop resistance after initial response to tamoxifen. To date, no effective targeted therapy exists to overcome it. We previously identified the role of nemo-like kinase (NLK), a serine-threonine kinase that functions in stress response and neurite outgrowth, in breast endocrine resistance. In addition, activation of p38 MAPK has been reported to modulate ER signaling and promote endocrine resistance. We identified a highly selective dual p38 and NLK kinase inhibitor (PNKI) through analysis of public kinase profiling datasets, and evaluated its therapeutic effect in endocrine-resistant breast cancers using in vitro and in preclinical mouse models. Experimental design and methods: To determine the in vitro therapeutic window of PNKI, we treated an acquired tamoxifen-resistant cell line (MCF7-TamR) and a benign breast epithelial cell line (MCF10A) with gradually increasing doses of PNKI. To determine the effect of PNKI on tamoxifen-resistant breast cancer cells, we treated primary tamoxifen-resistant breast cancer cell line BT483, and MDA-MB415, together with acquired tamoxifen-resistant line MCF7 TamR, T47D TamR, and ZR-75-B TamR, with 0.5 uM PNKI in the presence of different doses of Tamoxifen. To evaluate the therapeutic effect of PNKI in a T47D-derived xenograft tumor model with acquired tamoxifen resistance, we administered PNKI alone or in combination with Fulvestrant, the second-line endocrine therapy agent, or with Everolimus, the mTOR inhibitor that could improve patient outcomes in several clinical trials. Mice bearing xenografts were randomized into six treatment groups (Vehicle, PNKI, Fulvestrant, Fulvestrant+PNKI, Everolimus, Everolimus+PNKI). Tumor growth was tracked closely. The tumors harvested two weeks after treatments started were profiled with Reverse Phase Protein Array (RPPA) to assess the early signaling changes after treatments. The therapeutic effect of PNKI were also evaluated in a patient-derived xenograft (PDX) model of de novo endocrine resistant breast cancer. Mice bearing the PDX tumors were randomized to four treatment groups (Vehicle, PNKI, Everolimus, Everolimus+PNKI) and tumor growth curve was measured timely. Results: PNKI showed an in vitro therapeutic window at 0.1-1µM for MCF7-TamR cells. Breast cancer cell lines with either de novo or acquired Tamoxifen resistance became more sensitive to tamoxifen when treated with 0.5uM PNKI. The concomitant treatment of PNKI and Everolimus results in significant decreased tumor burden and prolonged progression free survival in the both T47D-TamR xenograft tumors and re-transplanted de novo endocrine-resistant PDX tumors compared to other treatments. RPPA data of T47D-TamR tumors harvested following 2-week treatments revealed that several key survival signaling in breast cancer are repressed only when PNKI are combined with Everolimus. Conclusion: The dual p38 and NLK inhibitor (PNKI) exhibited potential therapeutic value as adjuvant agent to the mTOR inhibitor everolimus for acquired or de novo tamoxifen-resistant luminal breast cancers.
Title: Bcl-2 functional converters inhibit tumor growth and metastatic potential in zebrafish xenografts

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Body: While potential therapies might have pronounced success in the simplified settings in cell culture medium, many drugs fail or underperform when cancer cells are encased in a complex 3D microenvironment. Although, rat and mouse models will continue to be the gold standard for in vivo data in drug discovery, zebrafish xenograft models have emerged as a powerful model that can quickly and efficiently deliver in vivo drug efficacy data before commitment to expensive and time consuming rodent models. We have discovered several compounds that work as B-cell lymphoma 2 (Bcl-2) functional converters and activate Bcl-2 into a killer instead of its native anti-apoptotic role. In this study, we use a zebrafish xenograft model to evaluate the ability of these compounds to inhibit xenograft tumor growth of Bcl-2 expressing cancer cells, including triple negative breast cancers. Live fluorescent imaging of cancer cells within zebrafish embryos revealed a decrease in cancer cell growth while under treatment of compounds. Furthermore, the agents that converted Bcl-2 into pro-apoptotic protein also inhibited the metastatic potential of the cancer cells. Therefore, this study demonstrates zebrafish xenograft techniques that can be used to quickly and efficiently obtain in vivo drug discovery data. Moreover, we report novel Bcl-2 functional converter compounds that can effectively reduce xenograft tumor growth and its ability to invade tissue in a living 3D environment and establish the role of Bcl-2 in cancer progression.
**Title:** Sensitivity of triple negative breast cancer cell lines to PCM-075, a highly selective polo-like kinase 1 inhibitor

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**Body:**

**Background:** The clinical adoption of targeted agents has vastly improved outcomes for subsets of breast cancer patients. Triple negative breast cancer (TNBC), however, remains a significant challenge due to its aggressive nature and lack of therapeutic options beyond chemotherapy. Polo-like kinases (PLKs), a family of serine-threonine kinases, regulate various cellular processes including mitosis, DNA replication, and stress response. Therapeutic targeting of PLK1 is of particular interest as it is over-expressed in many malignancies, including TNBC, and is required for progression through the G2/M phase of the cell cycle. PCM-075 is an orally-available, highly selective PLK1 inhibitor currently under clinical investigation. Our aim was to evaluate the activity of PCM-075, and related PLK inhibitors, in breast cancer cell lines.

**Methods:** We examined the activity of six PLK inhibitors (PCM-075, GSK461364, GW-843682X, BRD-K70511574, BI-2536, and Rigosertib) as measured by IC_{50} values across 40 publicly-available (Cancer Cell Line Encyclopedia, Broad Institute, Harvard, MA) breast cancer cell lines. PLK inhibitors were selected based on the availability of both IC_{50} and gene expression data for shared cell lines (Cancer Therapeutic Response Portal, Broad Institute). Statistically significant rank correlations between IC_{50} and normalized expression values for 18,541 genes were computed for each compound and across compounds by combining P-values. The relative expression status of the ERBB2, ESR1, and PGR genes was similarly assessed for each cell line.

**Results:** Hierarchical clustering based on the set of nominally significantly correlated genes (n = 350) revealed two clusters, suggesting that sensitivity to PLK inhibitors is determined by ESR1 status with positive (ER+) cell lines being insensitive. Further refinement of the cell line status according to ERBB2, ESR1, and PGR expression confirmed that ER- cell lines are the most sensitive to PLK inhibition. Among cell lines for which PCM-075 IC_{50} data was available, of 5 ER-/Her2+ cell lines, 2 were sensitive and 3 were insensitive. Of 17 TNBC cell lines, 14 (82%) were sensitive i.e. IC_{50} < 100 nM (PCM-075: Median ER+ IC_{50} = 1.000 mM; Median TNBC IC_{50} = 0.049 mM; P = 0.005). PCM-075 and GSK461364 exhibited similar sensitivity profiles and combining these data enhanced the distinction between ER+ and TNBC cell lines (PCM-075 and GSK461364: Median ER+ IC_{50} = 4.250 mM; Median TNBC IC_{50} = 0.049 mM; P = 0.0004). This was not the case for the remaining compounds combined (Median ER+ IC_{50} = 1.000 mM; Median TNBC IC_{50} = 0.409 mM; P = 0.018).

**Conclusions:** Examination of cell line response data across breast cancer cell lines indicates that ER- and specifically TNBC cell lines are exquisitely sensitive to PLK inhibition. Insufficient data was available to understand the relevance of Her2 status to this observation. Interestingly, the observed differential activity was pronounced for more selective PLK1 inhibitors. These findings suggest that this drug class may represent a viable therapeutic option and support further studies to assess the efficacy of PCM-075 in TNBC.
Human endogenous retrovirus TROJAN promotes triple negative breast cancer progression and represents a potential therapeutic target

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Body: Human endogenous retroviruses (HERVs) play pivotal roles in the development of breast cancer. However, the detailed mechanisms of noncoding HERVs remain elusive. We used RNA-Seq to analyze the transcription level of each HERV loci across the genome in eight pairs of triple negative breast cancer (TNBC) and normal breast tissues. We identified one LTR70, which we dubbed TROJAN, was a TNBC related HERV. We also found that HERVs could not be researched as families because each members from the same family had different expression patterns in TNBC. TROJAN promoted proliferation and invasion in TNBC cells and indicated poor outcomes in patients. Here, we confirmed that TROJAN regulated the progression of TNBC through multiple mechanisms, with ZMYND8, a metastasis-repressing factor, being one of the targets of TROJAN. TROJAN could bind to ZMYND8, promoting its degradation through ubiquitin-proteosome system, and downregulated the transcription level of EGFR, thus stimulating the metastasis of TNBC. The association of TROJAN, ZMYND8, and EGFR was subsequently confirmed in clinical samples. Furthermore, our study verified that the therapeutic delivery of antisense oligonucleotides targeting TROJAN significantly suppressed metastasis of TNBC in vivo. In conclusion, TROJAN promotes the progression of breast cancer and serves as a potential therapeutic target in TNBC.
Title: The BET bromodomain inhibitors ZEN-3694 and ZEN-3309 targets several mechanisms of resistance to endocrine therapies in preclinical models of ER+ breast cancers

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Body: More than 200,000 women are diagnosed with breast cancer every year in the United States. About 80% of these cases are estrogen receptor positive (ER+), which is characterized by the up-regulation of ER signaling and downstream activation of cyclin-dependent kinases CDK4/6 and cyclin D1 (CCND1). Current lines of therapies include either endocrine therapies or CDK4/6 inhibitors, which have resulted in great improvement in the treatment of ER+ breast cancer. As expected, resistance to these therapies occurs over time and the development of additional therapeutic strategies is needed. Recently, the bromodomain and extra terminal (BET) proteins BRD3 and BRD4 were shown to be involved in the transcription of ER, and BET inhibitor (BETi) treatment can suppress ER-mediated signaling, offering a potential strategy to overcome endocrine resistance by further shutting down ER signaling regardless of ESR1 mutation status. However, it is still unclear if BRD3/4 are involved in the mechanisms of resistance to endocrine therapies, and whether BETi can potently inhibit the proliferation of ER+ breast cancer cells that are resistant to CDK4/6 inhibitors.

ZEN-3694 is an orally bioavailable small molecule bromodomain BET inhibitor currently undergoing clinical development in metastatic castration-resistant prostate cancer (mCRPC). ZEN-3694 showed efficacy in a panel of ER+ breast cancer cell lines and synergized with both tamoxifen and fulvestrant to inhibit proliferation and induce apoptosis. ZEN-3694 also showed activity in several models of resistance to ER+ breast cancer therapies, including tamoxifen, fulvestrant, estrogen-deprivation, as well as with CDK4/6 inhibitors. Analysis of the major pathways that were up-regulated upon acquisition of resistance to endocrine therapies and down-regulated by ZEN-3694 revealed several key players previously involved in developing resistance to endocrine therapies in breast cancer patients, including: inflammatory cytokines (IL-6, IL-1A, IL-1B), inducers and regulators of epithelial-mesenchymal transition (EMT) (Slug and ZEB-1), cancer stem cell marker (CD44), and angiogenesis regulators (VEGFA, VEGFC). Therefore, several of the transcriptional programs linked to the acquisition of resistance to endocrine therapies are regulated by BET proteins and inhibited by ZEN-3694.

Together, these results suggest that BET inhibitors have the potential to be a novel therapeutic strategy to treat ER+ breast cancer patients developing resistance to endocrine therapies as well as CDK4/6 inhibitors.
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Title: Imipridone compounds inhibit breast cancer mTORC1 signaling through integrated stress response-mediated upregulation of endogenous mTORC1 inhibitor sestrin2

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Body: Breast cancer is a major cause of cancer-related death and there is a need for novel therapies with increased efficacy and decreased toxicity. The small molecule ONC201 was initially identified as a TRAIL pathway inducer. The compound has entered early phase clinical trials and is being tested in a range of solid tumors and hematological malignancies. Our previously published data demonstrate that ONC201 has potent anti-proliferative and pro-apoptotic effects in a broad range of breast cancer subtypes through TRAIL-dependent and TRAIL-independent mechanisms. Analogs of ONC201 with a shared pharmacophore have been developed and form the novel “imipridone” class. When compared with ONC201, imipridones ONC206, ONC212, and ONC213 showed increased potency of anti-proliferative or pro-apoptotic effects in breast cancer cells. We were interested in further defining the previously unstudied anti-proliferative effects of the imipridone compounds. Single agent efficacy of potent imipridone ONC212 in a xenograft model of TNBC was observed in the absence of apoptosis induction. This indicates that the anti-proliferative actions of the compound are sufficient for an in vivo anti-tumor effect. Our lab has previously shown that ONC201 activates an ATF4-dependent integrated stress response (ISR), essential for apoptosis induction in colon cancer cells. In contrast, in breast cancer cells, although ATF4 knockdown did not block cell death induced by ONC201 it did partially abrogate the anti-proliferative effects of the compound. The mammalian target of rapamycin complex 1 (mTORC1) is a well-known regulator of cellular growth and proliferation. mTORC1 signaling is inactivated in breast cancer cells following treatment with ONC201 and its analogs ONC212 and ONC213, regardless of whether the cells undergo apoptosis. Knockdown of ATF4 abrogated ONC201-mediated inhibition of p70 S6 kinase and ribosomal protein S6 phosphorylation, linking ISR induction to mTORC1 inhibition. We hypothesized that sestrin2, an endogenous mTORC1 inhibitor known to be upregulated following cellular stress, might represent a link between induction of ATF4 and inhibition of mTORC1. Treatment with ONC201 and its analogs ONC212 and ONC213 in multiple breast cancer cell lines resulted in sestrin2 upregulation. This was blocked by ATF4 knockdown. Furthermore, knockdown of sestrin2 abrogated the effects of ONC201 on mTORC1 signaling in breast cancers from multiple molecular subtypes. Previous mechanistic studies have focused exclusively on the relevance of ATF4 in the pro-apoptotic effects of ONC201. The novel findings described here help to elucidate the mechanism behind the potent and understudied anti-proliferative effects of the imipridones. Our findings also strengthen the preclinical rationale for testing of imipridone compounds against breast cancers regardless of molecular subtype.
**Title:** Discovery of novel oridonin-derivatives for the treatment of metastatic breast cancer

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**Body:** Metastasis to other organs is the major cause of death from breast cancer. The 5-year survival rate is approximately 99% for localized breast cancer, but sharply drops to approximately 26% for patients with distant metastasis. However, there are currently no effective, targeted therapies available for treating metastatic breast cancer. Oridonin, a complex *ent*-kaurene diterpenoid isolated from Chinese traditional herb *Rabdosia rubescens*, has demonstrated great potential in the treatment of various human cancers. However, relatively low aqueous solubility and bioavailability limited its development into clinical applications. Herein, a number of novel nitrogen-enriched heterocyclic oridonin derivatives and dienone analogs such as YD0514, CYD0618, and CYD0686 have been generated from oridonin. These derivative analogs showed improved anti-proliferation effects against breast cancer cells, compared to oridonin. YD0514, CYD0618, and CYD0686 also displayed significant suppression of migration and invasion in MDA-MB-231 cell, a highly metastatic triple-negative breast cancer cell line. Furthermore, YD0514, CYD0618 and CYD0686 also inhibited the endothelial adhesion of GI101 and its derived, highly metastatic sublines, to the endothelium. We next found that YD0514, CYD0618, and CYD0686 can significantly inhibit the expression and phosphorylation of FAK and block the expression of integrin family members in those highly metastatic breast cancer cell lines. Further pathway analysis demonstrated that YD0514, CYD0618, and CYD0686 inhibited cellular motility potentially by decreasing RHOA/ROCK signaling pathway. Our findings suggest that novel oridonin-derivatives, YD0514, CYD0618, and CYD0686 have the great potential to be developed as effective therapeutics for the treatment of metastatic breast cancer. This work was supported by Grants P50 CA097007, and P30DA028821 (JZ) from the NIH, CPRIT (JZ), John Sealy Memorial Endowment Fund (JZ), DFI Grants from MD Anderson Cancer Center (QS), and Holden Family Research Grant in BC Prevention (QS).
The impact of the Chinese medicine ShenLingLan on triple negative breast cancer, the metabolic and signalling pathways and clinical implications

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Body: Background. Triple negative breast cancer (TNBC) is a challenging both in the choice of therapies and clinical outcomes. In the present study, we investigated the potential prospect of a Chinese medicine formation, ShenLingLan, shown to have benefit to patients with cancer and able to influence the biological behaviour of cancer cells, on breast cancer cells in particular the differential response from TNBC and non-TNBC cells and, on the discovery that TNBC cells were particularly sensitive to the medicine, we went on to determine the signalling and mechanism of action.

Methods. A panel of three TNBC (MDA MB-231, BT20 and BT549) and a panel of three non-TNBC (MCF-7, ZR 75-1 and T47D) cells were used. A soluble extract from ShenLingLan, designated as SLDM, was utilised during this study. The proliferation, cellular migration and adhesiveness were tested using conventional and biophysical methods. Signalling profiling was conducted using a protein kinase array platform (Kinexus™). Metabolic profiling was conducted using the Seahorse platform. Expression of insulin receptor (IR) and insulin-like growth factor receptor (IGFR) gene transcripts (quantitative transcript analysis) and proteins (IHC) were conducted using a fresh breast cancer cohort and tissue array, respectively.

Results. SLDM had little effects on the growth of breast cancer cells. However, it had profound inhibitory effects on the migration of both TNBC and non-TNBC cells in a concentration dependent manner. Interestingly, TNBC cells were 5-20 times more sensitive than the non-TNBC cells in their migration and cell adhesion responses to SLDM. The protein array platform further revealed that, of the wide range of protein kinases, IR and IGFR1 were the most affected in that SLDM resulted in 25-50% reduction in the phosphorylation of IR and IR substrate in TNBC cells. SLDM also caused a contrasting response in IGFR1 phosphorylation in TNBC and non-TNBC cells. Metabolically, TNBC and non-TNBC cells responded to SLDM in very different fashions. For example, in TNBC cells SLDM resulted in a significant decrease in glycolytic activities, in particular that driven by insulin (30.2±12.2 pmol/min in control, 47.7±10 pmol/min with insulin and 35.4±4.2 pmol/min with insulin/SLDM, p=0.01). There is evidence that the mitochondria oxygen consumption (OCR) was also affected by SLDM in TNBC cells (p=0.01). These changes induced by SLDM were in clear contrast to non-TNBC cells which did not respond with significant reduction. Both TNBC and non-TNBC breast cancer tissues have higher IR staining than normal mammary tissues (p<0.001). TNBC tumours also demonstrated significantly more positive IR staining than the non-TNBC tumours (p=0.04).

Conclusion. ShenLingLan has a profound inhibitory effect on the migration and cell-matrix adhesion of TNBC cells, with marked effect on the metabolics of these cells. This effect connects with reduction of the IR and IGFR activation, mainly through the reduction in glycolysis. Together with the clinical implication of IR and IGFR in breast cancer, ShenLingLan has an important role in the treatment of breast cancer with an emphasis in TNBCs.
Title: IRS1 expression is required for estrogen stimulated growth in breast cancer cells

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Body: Insulin receptor substrate (IRS) proteins are adaptor proteins phosphorylated by activated type I insulin-like growth factor receptor (IGF-1R) and insulin receptor (IR). In addition to their roles in normal cell physiology, we have shown IRS expression is required for breast cancer cell growth and motility. IRS1 plays a more important role in mitogenesis and survival while IRS2 in cancer cell metastasis. Moreover, this family of adaptor proteins is also involved in the signal transduction of many other transmembrane receptors. Thus, IRS proteins could be potential cancer therapeutic targets. We have previously shown that reduced IRS1 impairs IGF and insulin stimulated cancer cell growth and tumorigenicity even when signaling is not affected due to possible compensation of IRS2 and other adaptor proteins. To further study the role of IRS-1 function in breast cancer, we created doxycycline inducible IRS-1 shRNA cells. We also found that IRS1 knockdown significantly reduced estradiol stimulated growth. To investigate if reduced IRS1 regulated E2 stimulated binding of ERα ChIP assay was performed using the pS2 promoter. Compared to parental MCF-7L cells, inducible IRS1 knock down clone 3G5 demonstrated significantly reduced promoter binding. Degradation of IRS-1 with a pharmacologic antagonist (NT-157) also diminished ERα binding to pS2 promoter. Further qRT-PCR analysis for mRNA levels of estrogen regulated genes, such as PGR, TFF1, etc. showed those genes were significantly down regulated. To evaluate effects of IRS-1 levels on estrogen stimulated growth, we evaluated 3G5-B12 (a subclone of 3G5) cell growth in a xenograft model. 3G5-B12 cells have similar levels of IGFIR and ERα expression compared to their parent cells. Estrogen dependent xenograft growth was similar to those of parent MCF-7L cells. After doxycycline was administered in the diet, 3G5-B12 tumor growth was significantly inhibited compared to those fed with normal mouse diet, and this inhibition was prolonged at least 2 and a half months. Parental MCF-7L tumor growth was not affected by the doxycycline diet. Doxycycline inducible IRS1 knock down significantly prolonged the time before tumors reached 1000mm³ compared with those tumors on a normal diet. In conclusion, IRS-1 is required for optimal estrogen receptor function as measured by promoter binding and xenograft growth. Our data suggested suppression of IRS-1 function may target several growth pathways in breast cancer cells and represents a new drug target.
Title: Inhibition of sushi domain containing 2 (SUSD2) cleavage prevents surface presentation of galectin-1 on breast cancer cells

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Body: Routinely used therapies are not adequate to treat the heterogeneity of breast cancer (BCa), and consequently, more therapeutic targets are desperately needed. Sushi Domain Containing 2 (SUSD2) is a type I transmembrane protein composed of functional domains inherent to adhesion molecules. SUSD2 is highly expressed in BCa but has minimal expression in other normal tissues. Using an in vivo syngeneic mouse model, the Egland lab demonstrated that mice with Susd2-expressing tumors showed increased tumor growth and decreased survival compared to vector controls. Interestingly, Susd2-expressing tumors contained 50% fewer infiltrating CD4 T cells when compared to vector control tumors. In vitro co-culture assays of MDA-MB-231 and Jurkat cells showed that MDA-MB-231-SUSD2 cells increased T cell death compared to MDA-MB-231-vector cells. Our preliminary experiments suggest that SUSD2 acts through the protein Galectin-1 (Gal-1) to achieve T cell killing. Gal-1 has been implicated as a major contributor to cancer immune evasion and angiogenesis. When Gal-1 is targeted by an inhibitory molecule, cancer progression is reduced. Surface presentation of Gal-1 by cancer cells was shown to be required for T cell killing. We have previously shown that localization of Gal-1 on the surface of cells is dependent on the presence of SUSD2; therefore, disrupting the SUSD2-Gal-1 interaction offers a potential new therapeutic strategy for BCa. Western immunoblot analysis using an anti-SUSD2 antibody against the C-terminus showed that SUSD2 runs as two bands, a 110kDa full-length band and a 60kDa fragment, suggesting that SUSD2 is cleaved. When antibodies against both the C-terminus and N-terminus of SUSD2 were used, three bands were observed: a full-length 110kDa band and fragment bands of 60kDa and 50kDa. The cleavage site and significance of this cleavage with respect to SUSD2 and Gal-1 interactions previously has not been investigated. Edman degradation was used to sequence the C-terminal SUSD2 fragment and determined that the cleavage site is within the Von Willebrand type D domain, between the aspartic acid and proline of the GDPH sequence. Deletion of the GDPH sequence showed a complete inhibition of SUSD2 cleavage. Furthermore, non-cleaved SUSD2 was unable to localize to the plasma membrane indicating that SUSD2 cleavage is important for cell surface presentation. Consistently, Gal-1 was not localized to the cell surface in MDA-MB-231 cells expressing the non-cleavable SUSD2 mutant. Our results indicate that SUSD2 cleavage is a processing step required for Gal-1 surface localization and inhibiting SUSD2 processing may be a viable targeting strategy. We are treating SUSD2-expressing BCa cells with protease inhibitors to identify the protease responsible for SUSD2 cleavage. The identification of this protease would provide a potential target for decreasing BCa tumorigenesis and increasing survival of BCa patients.
Body: Under physiological conditions low voltage-activated calcium channels (LVA, also called T-type channels) occur mostly in the brain, peripheral nervous system, smooth muscles, and pacemaker of the heart. However, LVA are often aberrantly expressed in various cancers and their overexpression is inversely correlated with outcomes. Active LVA contribute to proliferation, progression and resistance to therapy in several human cancers, including breast cancer (BC). The molecular mechanism of this activity is still not fully understood, and there is a lack of pre-clinical studies that target LVA in animal models of BC. Data from the Cancer Genome Atlas (TCGA) show a positive correlation between HER2 gene amplification and LVA channel overexpression, particularly of Cav3.1 (CACNA1G) subunit. The goal of this study is to identify functional links between HER2 receptor signaling and LVA channels, and validate LVA Ca\textsuperscript{2+} channels as a potential target for BC therapy. Inhibition of LVA, by small molecule antagonist mibefradil (MIB) or shRNA, significantly decreased the activity of PI3K/mTOR/AKT pathway in HER2-positive BC cell lines, blocked cell cycle progression, and at higher concentrations induced apoptotic cell death. Furthermore, LVA antagonist, mibefradil, sensitized cells to trastuzumab, lapatinib and paclitaxel, commonly used standard treatments for HER2-positive BC. To test the effects of LVA inhibition in vivo, the MMTV-PyMT mouse model of BC was used. In this model several features of HER2-positive BC are recapitulated, such as activation of PI3K/AKT/mTOR pathway, overexpression of HER2, BIRC5 and cyclin D1, and low expression of ER\textalpha, PGR and FOXA1. In PyMT animals palpable tumors can be detected at the age of 10-12 weeks, and 100% of animals will develop high tumor burden, including lung metastases, at the age of 15-20 weeks. Expression of CACNA1G subunit was detected in breast epithelial cells of 7 week old mice, and throughout the adult life, in both wild-type and PyMT animals, but CACNA1G was not expressed in lung or skeletal muscles. In in vitro experiments, cell lines derived from PyMT tumors were sensitive to MIB. In in vivo experiment, 5 to 7 weeks-old PyMT mice were fed either control or MIB-containing diet ad lib. Analysis of mouse serum revealed stable MIB concentration of \~1500 ng/mL achieved within one week of treatment. In MIB-fed animals tumor appearance was delayed by 2 weeks, and the tumors were smaller as compared to control, thus suggesting a supporting role for LVA channels in breast tumor development and progression. Together, our observations provide new insights into the role of Ca\textsuperscript{2+} channels in HER2 driven BC and posit a future use of LVA channel antagonists for the treatment HER2-positive breast tumors.
Purpose: Breast tumors classified as ‘triple negative’ (TNBC) lack defining markers ER/PR/HER2 and do not have clinically-approved targeted therapy. This heterogeneous classification of breast cancers, while immediately responsive to standard chemotherapy, commonly develop resistance and have a poor five-year survival rate. As such, the identification of new therapeutic targets are warranted. As part of our drug discovery platform, we have identified EphA2, as a synthetic-lethal gene that enhances the therapeutic action of FDA-approved, anti-inflammatory compounds. Thus we sought to ascertain the relevance of EphA2-targeted therapy in TNBC, through the evaluation of the marker in preclinical and clinical specimens.

Methods: Sixty-one human and murine breast cancer cell lines or patient-derived xenografts were collated. Protein lysates were created from cells in vitro or from respective tumors established from cells implanted into NSG mice. Forty-nine tumors established (minimum 500mm$^3$) and were surgically removed, fixed in formalin and paraffin embedded. A TMA was constructed with tumor specimens represented twice on the array and reflected all molecular subtypes including; ER-positive (n=5), PR-positive (n=3), HER2-positive (n=9) and TNBC (n=31). Immunostaining for EphA2 was performed with the rabbit monoclonal antibody EphA2 (D4A2) XP (Cell Signaling, #6997) using manufacturer's instructions. Immunostaining was evaluated using the H-score method (score between 0-300), with positive staining for EphA2 reflecting a score of 100 or greater. Analysis of breast cancer lysates by western blot was analyzed by absolute and relative quantitation methods; gene expression data was assessed through Oncomine or using the BreastMark algorithm (http://glados.ucd.ie/BreastMark/). This algorithm integrates gene expression and survival data from 26 datasets on 12 different microarray platforms corresponding to ~17,000 genes in up to 4,738 samples.

Results: In an integrated gene expression platform (BreastMark), we observed that elevated EphA2 expression was associated with poor prognosis in a cohort of TNBC patient tumor samples. Western blot analysis of EphA2 protein on breast cancer cell lines, identified a greater percentage of TNBC cells expressing EphA2 compared to non-TNBC cell lines. EphA2 immunostaining was observed in the majority of tumor tissues. When present on cancer cells, EphA2 localized to the cell surface; while displaying ubiquitous localization within stromal populations. Cell surface expression of EphA2 on cancer cells was largely restricted to TNBC tumors (11/31 tumors, 35.5%) compared to other molecular subtypes (1/13 non-TNBC tumors, 7.7%; p = 0.0294). Expression of EphA2 in stromal cell populations was similar between groups (TNBC = 22/31, non-TNBC = 11/13; p = 0.1711).

Conclusions: Our analysis determined that EphA2 was specifically expressed on cancer cells derived from tumors with a ‘triple-negative’ molecular subtype. Collectively our data suggests that EphA2 is an emerging target in TNBC and that therapies directed against EphA2 may provide a significant benefit for a majority of patients that express this marker.
Potential of breast tumour kinase (Brk) as a therapeutic target: Brk modulates drug responses in breast cancer cell lines

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**Body: Background:** Breast tumour kinase (Brk/PTK6) is over-expressed in up to 86% of all breast cancers and has shown to be involved in the processes regulating tumour development and progression. A few Brk inhibitors are in development and our studies indicate potential for a Brk inhibitor to be used in combination with current breast cancer therapies. Furthermore the effect of common breast cancer therapies and Brk inhibition on the expression of \( ptk6 \) as well as its alternatively spliced isoform (ALT-PTK6) have not been previously been shown. Using qPCR we have determined gene expression of both genes before and after treatment.

**Methods:** Breast cancer cell lines T47D, GI101, BT474, SKBR3, MDA-MB 231, and MDA-MB 436 were treated with Brk inhibitor, Compound 4f (Mahmoud et al, 2012) and common breast cancer therapies (Taxol, Doxorubicin, Lapatinib and Tamoxifen) at concentrations ranging from 0µM to 10µM. Western blotting was carried out to determine levels of Brk and activation of Brk substrate; STAT3 after treatment with Brk inhibitor. RNA was extracted using Qiagen Mini RNase prep kit, cDNA synthesized using Invitrogen Superscript II Reverse Transcriptase and qPCR carried out using primers specific for PTK6 or primers that recognized both transcripts (PTK6 and ALT-PTK6) for total expression.

**Results:** In all cell lines tested there was a moderate reduction in cell proliferation following treatment with the Brk inhibitor (4f). However a greater effect was observed in combination therapy. Triple negative breast cancer cell lines MDA-MB 231 and MDA-MB 436 were treated with Compound 4f and Taxol or Doxorubicin (n=3) resulting in modest but statistically significant reduction in cell numbers. Cell responses to Taxol in both cell lines were significantly greater in the presence of 4f over a range of doses (\( P < 0.05 \) and \( P < 0.007 \)). Responses to Doxorubicin were also significantly improved in the presence of 4f (\( P < 0.03 \) for MDA-MB-231 and \( P = 0.03 \) for MDA-MB-436). Co-treatment of HER2 positive breast cancer cell lines BT474 and Sk-Br-3 with Lapatinib and 4f showed significant increase in responses over a range of doses between 0.31 and 5µM (n=3, \( P < 0.05 \) for BT474 and \( P \) between 0.03 and 0.0004 for Sk-Br-3). In comparison to untreated cells, there was statistically significant reduction in \( ptk6 \) and Total gene expression observed at various time points within multiple breast cancer cell lines in response to Compound 4f treatment. Significant differences between untreated and treated cells for T47D cell line were at 8 hours post treatment (\( p = 0.02 \)), 2 and 4 hours post treatment in GI101 cell line (\( p=0.04 \) and \( p=0.02 \) respectively), 8 and 24 hours post treatment in Sk-Br-3 (\( p=0.001 \) and \( p=0.017 \) respectively) and 8 hours post treatment in MDA-MB 231 cell lines (\( p=0.03 \)).

**Conclusion:** Inhibition of Brk led to a decrease in \( ptk6 \) gene expression. Expression ratios of \( ptk6 \) and the short isoform ALT-PTK6 were determined and a reduction of \( ptk6 \) gene correlated with elevation of ALT-PTK6 and vice versa. Inhibition of Brk also indicated an increase in breast cancer cell sensitivity to current breast cancer therapies. Our studies thus indicate potential for inhibition of Brk kinase activity in combination with current breast cancer treatments.
Title: TRIM44 is a possible poor prognostic factor for breast cancer patients and positively regulates NF-κB signaling pathway

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Body: [Background]
Many of the tripartite motif (TRIM) proteins, like Efp/TRIM25 which was identified by our group previously (Nature 417, 871-875, 2002), function as E3 ubiquitin ligases, and are thought to be involved in various physiological and pathological processes such as immunity and oncogenesis. In regard to tripartite motif containing 44 (TRIM44), which is an atypical TRIM family protein lacking RING finger domain, some evidences suggest that it is implicated in the progression of several human malignancies. But its pathophysiological significance in breast cancer remains unknown.

[Methods]
In the present study, immunohistochemical analysis using anti-TRIM44 antibody was performed in clinical breast cancer tissues from 129 patients with the approval of institutional ethical committees (approval number: 845). We then explored the pathophysiological role of TRIM44 in breast cancer by modulating TRIM44 expression in MCF-7 and MDA-MB-231 breast cancer cells.

[Results]
TRIM44 strong immunoreactivity was significantly associated with nuclear grade, distant disease-free survival and overall survival of the breast cancer patients. With multivariate analysis it was shown that the TRIM44 status was an independent prognostic factor for distant disease-free survival and overall survival. The proliferation of MCF-7 and MDA-MB-231 cells was significantly decreased by siRNA-mediated TRIM44 knockdown. TRIM44 knockdown also suppressed migration of MDA-MB-231 cells. Microarray analysis and qRT-PCR revealed that TRIM44 knockdown upregulated CDK19 (Cyclin Dependent Kinase 19), which is reported to be a tumor suppressor gene, whereas downregulated MMP1 (Matrix Metallopeptidase 1) in MDA-MB-231 cells. Notably, TRIM44 knockdown impaired nuclear factor-kappa B (NF-κB)-mediated transcriptional activity stimulated by tumor necrosis factor α (TNFα). Moreover, TRIM44 knockdown substantially attenuated the TNFα-dependent phosphorylation of p65 subunit of NF-κB and IκBα in both MCF-7 and MDA-MB-231 cells.

[Discussion]
Our clinical study showed that prognosis of breast cancer patients is correlated with the immunoreactivity detected by anti-TRIM44 antibody. This result suggested that expression of TRIM44 protein could be used as a potential biomarker of breast cancer. We demonstrated that NF-κB signaling pathway is modulated by TRIM44. Since NF-κB augmentation is shown to be related to aggressive character of breast cancer, stimulation of NF-κB signaling with TRIM44 might be underlying mechanism of poor prognosis. Our in vitro study showed TRIM44 knockdown caused attenuated proliferation and migration of breast cancer cells, raising the possibility of TRIM44 as a potential therapeutic target for breast cancer. These findings provide new clues to develop alternative effective strategies for breast cancer management.
Title: The pan-HER inhibitor, neratinib and wingless-type MMTVs (Wnt)/Wnt regulators in human breast cancer; a biological and clinical perspective

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Body: Background. Neratinib is an orally available tyrosine kinase inhibitor that irreversibly binds and inhibits EGFR, HER2 and HER4 receptor tyrosine kinases. Neratinib has been shown to have clinical activity in HER2-amplified or overexpressed breast cancers and those with HER2 mutations. However, there are indications that it may also work on other subtypes that are not strongly positive for the receptors. The present study first screened the effects of neratinib on a range of kinase targets and identified that the Wnt signalling components are key factors that allow neratinib to interact and targets. These targets were also validated in a cohort of human breast cancer.

Methods. Human breast cancer cohorts (n=124) were tested for the transcript expression of HER family including EGFR, HER2, HER3 and HER4 and a number of Wnt family members and the Wnt signalling regulators, ie. GSK3, Axin-1, Axin-2 and β-catenin. The expression patterns were analysed against the clinicopathological and survival status of the patients. Neratinib was tested on a panel of breast cancer cell lines including triple negative cells for the effects on cytotoxicity, cell growth, matrix adhesiveness and cellular migration. Signalling kinase pathways were screened using an antibody based kinase array. The effect of neratinib on multiple protein kinases was tested on the cell models, together with other kinase inhibitors.

Results. Neratinib had an inhibitory effect on the cellular migration and cell-matrix adhesiveness of breast cancers at non-toxic concentrations, an effect more profound with MCF-7 and T47D cell lines than with BT20 and MDA MB-231 which are negative for the ER/EGFR/HER2 receptors. Of the multiple kinase inhibitors tested, neratinib was found to exert inhibition on cell function in synergy with the Wnt/β-catenin inhibitor (FK535) and GSK3 inhibitor (TWS119). The expression of the HER and Wnt family members, Wnt Inhibitory Factor-1 and Wnt regulators varied in mammary and breast cancer tissues and in their correlation with the clinicopathological factors. Of the aberrantly expressed receptors, Wnts and Wnt regulators, HER-2 and 4 were found to significantly correlate with Wnt10b (p<0.05), EGFR/HER1 was found significantly correlated with GSK3 (p<0.05) and HER-3 with Wnt5a (p<0.03). Furthermore, we identified that the integrated expression pattern of five of these factors, namely EGFR, HER2, HER4, Wnt5 and Wnt Inhibitory Factor-1 formed an expression signature that were significantly linked to the overall survival (survival time 148±3.7 vs 113.7 ±7.5 months for favourable and non-favourable pattern, respectively, p=0.002) and disease free survival (p=0.004) of the patients (median follow-up 120 months)

Conclusion. Neratinib, at non-toxic concentration levels, is a profound inhibitor of the migration and matrix adhesion of breast cancer cells, cell functions linked to the aggressiveness and metastasis of breast cancer cells. Its synergistic effects with the Wnt and GSK3 inhibitor, together with the prognostic value of the HER family/Wnt, indicate that the Wnt pathway together with the HER family forms a new molecular indicator and target when considering neratinib in the treatment patients with breast cancer.
Title: Temozolomide as a targeted therapy strategy for triple negative breast cancer

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Body: BACKGROUND:
Approximately 15% of newly diagnosed breast cancers are classified as triple negative (TNBC). TNBCs are considered more aggressive and have a worse prognosis as no targeted therapies are currently available. These tumors are routinely treated with chemotherapy agents with only modest proven efficacy.

Temozolomide (TMZ) is an oral chemotherapy agent commonly used for the treatment of brain tumors and melanoma. TMZ is an alkylating agent, and its therapeutic benefit depends on its ability to alkylate/methylate DNA, most commonly at the N-7 or O-6 positions of guanine residues. This process leads to DNA damage and subsequently triggers cell death. Cells that express the enzyme O⁶-Methylguanine-DNA Methyltransferase (MGMT) are able to repair damage caused by TMZ. Tumors that lack expression of MGMT, owing to methylation of the gene promoter, demonstrate a better response to TMZ treatment as a result of synthetic lethality.

It was first reported in 2012 that TNBCs were more likely to be MGMT methylated, which was confirmed by another group that reported up to 64% of wild-type BRCA1 TNBC exhibited MGMT gene methylation. In 2013 it was found that basal-like breast cancers were more likely to be MGMT methylated and linked to larger tumor size. Together these findings suggest that a sub-population of TNBCs lack MGMT expression, due to promoter methylation.

Currently, TMZ is not a treatment option for breast cancers given the modest efficacy of TMZ noted in breast cancer clinical trials; however, most of these trials have focused on using this agent to either treat or prevent brain metastases, due to TMZ's ability to cross the blood-brain barrier. Importantly, none of these trials investigated MGMT expression or specifically TNBC populations.

We hypothesize that TMZ may be a viable and efficacious treatment option for TNBCs that lack MGMT expression, due to promoter methylation.

METHODS:
We analyzed 12 archival specimens and 4 TNBC cell lines (HTB132, HTB26, HTB126 and HCC1806) for MGMT expression using a qRT-PCR clinical assay available from Calgary Laboratory Services. Additionally, we also looked at MGMT protein expression in the cell lines using Western Blot analysis to confirm the qRT-PCR results. Finally, we performed an in vitro assay with TNBC cell lines to determine cytotoxicity of TMZ.

RESULTS:
Analysis of the archival specimens found that 33% of samples analyzed had MGMT promoter methylation by qRT-PCR. Additionally, we found that HTB26 and HTB126 cell lines showed MGMT promoter methylation by qRT-PCR analysis. Western Blot analysis confirmed lack of MGMT expression in these two cell lines, and also identified another cell line (HCC1806) lacking MGMT protein that was classified as unmethylated by the qRT-PCR clinical assay. Moreover, our in vitro assay found that two cell lines (HTB26 and HCC1806) showed a noticeable response to treatment with TMZ. Interestingly, HTB126 did not show response to TMZ, suggesting that there may be another putative resistance pathway.

CONCLUSIONS:
Preliminary findings suggest that TMZ may be a viable targeted treatment option for TNBCs. Currently, we are investigating drug response using in vivo mouse models, as well as investigating synergistic combination therapy options.
**Title:** ERRβ copy number and expression in triple negative breast cancer

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**Body:**

**Introduction:** Triple negative breast cancer (TNBC) is a highly aggressive form of breast cancer prevalent in African-American (AA) women defined as estrogen receptor- (ER), progesterone receptor- (PR), and human epidermal growth factor receptor 2- (HER2) negative. Because ER- and HER2-targeted therapies are ineffective in TNBC, systemic chemotherapy is the standard of care and there is a tremendous need for new effective therapies with less toxicity. Steroid hormone receptors are highly druggable targets, and orphan nuclear receptors, members of the nuclear receptor superfamily, are emerging as targets for cancer therapy. In fact, we have previously shown that treatment of TNBC cells with a small molecule agonist ligand (DY131) for estrogen related receptor beta (ERRβ), has growth inhibitory and anti-mitotic activity. We have also shown that increased mRNA expression of ERRβ, correlates with better recurrence- and distant metastasis-free survival in TNBC/basal-like breast cancer. The goal of our current work is to comprehensively characterize ERRβ copy number and mRNA status in TNBC and determine its association with patients’ prognosis.

**Methods:** ESRRB copy number was determined in 106 primary breast tumors (TNBC n=56, nonTNBC n=50) by array-CGH, using the Agilent SurePrint G3 Human CGH platform. ESRRB mRNA data and its association with overall survival was determined in systemically untreated patients from METABRIC using Illumina gene expression array data (probe ID ILMN_1707398).

**Results:**

**Copy number alterations (CNAs).** Copy number losses at the ESRRB locus (14q24.3) were observed in 10/56 (17.8%) of TNBC vs. 10/50 (20%) of nonTNBC, while copy number gains were detected in 43/56 (76.8%) of TNBC vs. 29/50 (58%) of nonTNBC (c² *p=0.036). Interestingly, in both TNBC and non-TNBC, ESRRB loss was seen with markedly higher frequency in AA patients when compared to Caucasian (CA) patients (c² *p=0.012 for TNBC, p=0.052 for non-TNBC).

**mRNA expression.** Among patients not treated with systemic chemotheraphy in the METABRIC dataset, low ESRRB mRNA was significantly associated with shorter overall survival in TNBC, but not ER+ or HER2+ patients (TNBC hazard ratio 0.24, 95% confidence interval 0.07-0.85, *p=0.016). Low ESRRB also correlated with reduced overall survival in TP53 mutant (but not wild type) tumors (hazard ratio 0.28, 95% confidence interval 0.1-0.82, *p=0.013).

**Conclusions:** ESRRB presents significantly high levels of copy number losses in TNBC when compared to non-TNBC tumors. In breast tumors from AA women, both the TNBC and non-TNBC subtypes are significantly more likely to have reduced ESRRB copy number vs. CA women. Low ESRRB mRNA expression predicts for poor overall survival in TNBC and TP53 mutant tumors. These data advocate that ERRβ expression has prognostic value in breast cancer, particularly TNBC. Future goals include immunohistochemistry staining, and analysis, of a tissue microarray consisting of 150 primary breast tumors (50 TNBC, 50 ER+, 50 HER2+); as well as ERRβ overexpression and knock-down studies in TNBC cell lines to define the role it plays in TNBC.
Title: Modulation of FASN under obese conditions

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Body: Introduction: Obesity is known to be associated with a worse breast cancer prognosis, in part through altering metabolism in cells of the tumor microenvironment. In particular, changes in metabolism associated with fatty acid utilization have been noted in not only breast cancer, but also several other cancer types. This includes changes to both expression and activity of the Fatty Acid Synthase enzyme (FASN), which is responsible for production of long chain fatty acids, including palmitate. These changes in long chain fatty acid production can modulate tumor behavior through modulation of energy utilization such as beta-oxidation, as well as plasma membrane modulation with phospholipids. Our previous studies have demonstrated that exposure to obese conditions induces significant changes in breast cancer cell proliferation. Additionally, obesity modulates activity of other cells within the tumor microenvironment, including adipocytes, which might influence the cancer cell itself. We hypothesize one particular mechanism that supports these changes is obesity-induced upregulation of FASN and that FASN may be a viable target to limit obesity-induced progression.

Methods and Results: FASN has been shown to promote cancer cell proliferation through generating fatty acid precursors required for cell proliferation, altering membrane fluidity, and activating oncogenic signaling pathways. To determine if modulation of FASN is an important mechanism by which obesity promotes disease progression, MCF-7 breast cancer cells and human pre-adipocyte cells (ASC) were exposed to 2% sera from obese postmenopausal women and 2% sera from non-obese (control) women. Preliminary quantitative PCR results demonstrated that exposure to the obese sera resulted in increased expression of FASN in both the cancer cells as well as the ASC. Current studies are on-going to determine if 1) FASN up-regulation results in increased long-chain and free fatty acid production in both the cancer and adipocyte cells, 2) whether changes in long chain and free fatty acid production results in altered metabolism and plasma membrane status and 3) whether targeting FASN with a new generation of FASN inhibitors currently being investigated in the clinic can modulate obesity-induced disease progression.

Conclusions: Our findings indicate that obesity promotes upregulation of FASN in several cells within the tumor microenvironment, including adipocytes and the cancer cell itself. We have also found that using a FASN inhibitor is effective in limiting cancer cell viability and proliferation. Our on-going studies will confirm if this is an important mechanism by which obesity promotes disease progression. Since FASN inhibitors are currently being investigated in the clinic, the results of these studies will provide a better understanding of how obesity alters the biology of the disease, and may identify a novel target for improving patient outcomes.
Body: Background: Invasive lobular carcinomas (ILC) account for around 15% of all oestrogen receptor (ER) positive invasive breast cancers. The EndoPredict assay (EPclin) is a multigene classifier to predict the likelihood of distant recurrence in ER-positive, HER2-negative breast cancer patients treated with adjuvant endocrine therapy and has been validated in several retrospective translational studies. However, these validation studies did not include an analysis of the histological subtypes. Here, we investigate the role of EPclin for the prediction of distant recurrence in women with ILC and compare the prognostication to those with invasive ductal carcinoma (IDC) in TransATAC.

Methods: Data on EPclin and histological type of tumour were available for 928 postmenopausal women with ER-positive, HER2-negative disease treated with 5 years of tamoxifen or anastrozole. The primary endpoint was distant recurrence and the primary objective was to assess the prognostic value of EPclin for the prediction of distant recurrence in women with invasive lobular carcinoma. Kaplan–Meier and Cox regression analyses were used to determine distant recurrence risk for 0-10 years of follow-up. Likelihood ratio tests were used to assess the prognostic information provided by EPclin. Hazard Ratios (HR) are for a change in 1 Standard Deviation.

Results: 141 (15.2%) had ILC, 710 (76.5%) IDC, and the remaining 77 (8.3%) were of different histological type. EPclin provided highly significant prognostic value for distant recurrence in women with ILC (HR=3.33 (2.33-4.77), P<0.001; LR-χ²=38.4). For those with IDC, EPclin was highly prognostic for distant recurrence over 10 years of follow-up (HR=2.41 (2.04-2.84), P<0.001; LR-χ²=95.5). EPclin was more prognostic in IDC than ILC, and a significant interaction between EPclin and tumour type (ILC/IDC) was observed (P-interaction=0.038). 89 (63.1%) women with ILC were categorised into the low EPclin risk group and 52 (36.9%) into the high risk group. A highly significant separation between EPclin low and high risk groups was observed for ILC (10-year distant recurrence risk low: 6.2% (2.6-14.2) vs. high: 36.6% (24.2-52.1); HR=7.36 (2.71-20.01)). For women with IDC, 406 (57.2%) were categorised into the low risk group by EPclin and 304 (42.8%) were deemed high risk, with significant separation between risk groups (10-year distant recurrence risk low: 6.2% (4.1-9.3) vs. high: 28.5% (23.5-34.3); HR=5.59 (3.48-8.98)). We did not observe any differential efficacy of treatment between histological subtype and EPclin risk group.

Conclusions: This is the first analysis to focus on the role of EPclin for the prognostic assessment of women with ILC. Although numbers of women with ILC in TransATAC were small, EPclin provided highly significant prognostic information and risk stratification for this subgroup of women. 10-year distant recurrence risk in the EPclin low risk groups were similar between ILC and IDC, suggesting that chemotherapy is not indicated, irrespective of tumour type. Our results show that EPclin is informative in women with ILC.
Title: Validation of the online prediction tool PREDICT v. 2.0: A large population-based study in the Netherlands

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Body: Background
This study aimed to validate the online prediction tool PREDICT version 2.0 on a large population-based cohort in the Netherlands. Separate analyses were performed to study its validity in specific prognostic subgroups.

Methods
All women diagnosed with non-metastasised primary invasive breast cancer in 2005, who received surgery as part of their treatment, were selected from the nationwide Netherlands Cancer Registry (NCR). The predicted 5-year and 10-year overall survival (OS) from PREDICT were compared with the observed OS from the NCR, for the overall cohort (separated by ER- and ER+ patients) and for predefined subgroups based on age, T stage, N stage, presence of micrometastases, grade, HER2 status, type of surgery, use and type of adjuvant systemic therapy and generation chemotherapy. Our a priori assumption was that PREDICT accurately predicted OS whenever the differences between predicted and observed outcomes were within a range of 5%, since a difference of 5% or more was considered as clinically relevant. The discriminatory accuracy and goodness-of-fit were determined using the area under the receiver operating characteristic curve (AUC) and the Chi2-test, respectively.

Results
Our study population consisted of 8,834 patients. Discriminatory accuracy for 5-year OS was good with an AUC of 0.80. For ER- and ER+ patients, the AUCs were 0.75 and 0.79, respectively. Predicted 5-year OS differed from observed by -1.4% (p=0.14) in the entire cohort, by -4.9% (p=0.02) in ER- patients and -0.7% (p=0.53) in ER+ patients. In all predefined subgroups no statistically significant differences between predicted and observed events were observed.

Discriminatory accuracy for 10-year OS was good with an AUC of 0.78 for the overall cohort, 0.76 for ER- patients and 0.78 for ER+ patients. Predicted 10-year OS differed from observed by -1.0% (p=0.27) in the entire cohort, by -5.3 (p=0.01) in ER- patients and -0.1% (p=0.92) in ER+ patients. Ten-year OS was overestimated in patients ≥75 years (6.3%, p=0.00), and underestimated in T3 stage (-13.%, p=0.00) and in patients treated with both endocrine therapy and chemotherapy (-6.6%, p=0.02).

Conclusion
PREDICT accurately predicts 5-year OS in the entire Dutch validation population, and in all predefined subgroups. However, 10-year OS was overestimated for patients ≥75 years, and underestimated for T3 stage and patients treated with both endocrine therapy and chemotherapy. Given PREDICT’s intentions to guide treatment decision-making, PREDICT may serve as a reliable prediction tool for the Dutch breast cancer population, but especially in patients ≥75 years, 10-year OS predictions should be interpreted with care.
Title: Validation of a nomogram for predicting recurrence among women with ductal carcinoma in situ and breast conserving surgery in an integrated health care system

Michael C Leo¹, Melanie Francisco¹, Charisma Jenkins¹ and Sheila Weinmann¹. ¹Kaiser Permanente Center for Health Research, Portland, OR.

Body: Current management of ductal carcinoma in situ (DCIS) most often entails removing the lesion by breast-conserving surgery. Quantifying the risk of a patient's ipsilateral breast event (IBE) recurrence, either invasive cancer or DCIS, after breast conserving surgery remains a clinical concern. The aim of this study was to validate the Memorial Sloan-Kettering Cancer Center (MSKCC) nomogram to predict IBE recurrence in patients from our institution.

Patients and Methods
We retrospectively identified 608 patients in the Kaiser Permanente Northwest integrated healthcare system with a diagnosis of DCIS who had undergone local excision from 1990 through 2007. We assessed the performance of the MSKCC nomogram for predicting IBE recurrence using measures of discrimination (how well risk scores separate those with and without the event) and calibration (agreement between predicted and observed risk). We calculated Harrell's C and R² to provide estimates of discrimination. We calculated the calibration slope by performing a Cox regression using the prognostic index (PI; predicted log relative hazard based on the original Cox coefficients), with a slope not significantly different from 1 indicating no difference in discrimination from the development sample. We also examined discrimination by comparing the KM curves of 4 risk groups and in a Cox regression as a predictor, which were created using Cox's method (4 groups using the 16th, 50th, & 84th percentiles), as no risk groups were defined in the MSKCC development study. We examined whether there was model misfit by testing each predictor in a Cox regression with an offset of the PI. Finally, we assessed calibration by plotting the observed rates and associated 95% CIs against the predicted probabilities for groups based on 4 risk groups and octiles.

Results
The median follow-up time for the KPNW cohort was 125 months. The 10-year IBE recurrence rate was 9.5%, 95% CI [7.0%, 13.0%). Harrell's C was .70, which is comparable to what has been found in other validation studies. The PI accounted for 22% of variation in time (R² = .22, 95% CI [.08, .38]). The test of the calibration slope provided no support that discrimination in this sample differs from the development study (LR χ²(1)=0.21, p=.65). An examination of the Kaplan-Meier curves among the risk groups showed good separation of the high risk group compared to the others, but little separation between the lowest two risk groups. None of the predictors demonstrated evidence for differential weighting from the MSKCC coefficients (p values ranged from .08 to .97). Calibration was good for the lowest, low, and moderate risk groups, but there was underprediction in the high risk group. When examining calibration using octiles, we found a similar pattern to that of other validation studies in which the highest octile had the furthest departure from perfect agreement.

Conclusion
The MSKCC nomogram for predicting IBE recurrence in patients with DCIS who were treated with local excision have some utility, and our results are consistent with other validation efforts. However, there is much potential to further increase the prediction of recurrence beyond what is possible with the MSKCC nomogram.
Title: Prognostic value of OncoMasTR: A novel multigene signature based on master transcriptional regulators

Stephen Barron¹, Karin Jirström², Helena Jernström², Christian Ingvar², Bruce Moran¹,³, Chan-Ju Angel Wang¹, Tony Loughman¹, Bozena Fender¹, Peter Dynoodt¹, Cesar Lopez-Ruiz¹, Niamh Russell³ and William M Gallagher¹,³.¹ OncoMark Ltd, Dublin, Ireland; ² Lund University and Skåne University Hospital, Lund, Sweden and ³ School of Biomolecular and Biomedical Science, Conway Institute, University College Dublin, Dublin, Ireland.

Body: Background
Multigene prognostic signatures (MGPS) enable identification of candidate patients for treatment de-escalation in early stage breast cancer (BC). Here we present OncoMasTR, a MGPS for classifying the risk of distant metastasis (DM) in ER-positive, HER2-negative BC patients with up to 3 involved lymph nodes (LNs). OncoMasTR was discovered via a novel transcriptional network analysis methodology that identified genes that regulate previously identified prognostic biomarkers. These upstream genes, termed master transcriptional regulators (MTRs), were shown to provide improved prognostic performance compared with downstream genes. OncoMasTR has been mechanistically verified by RT-qPCR, immunohistochemistry and chromatin immunoprecipitation. OncoMasTR has been further trained to include clinicopathological information (CPI) to maximise its prognostic performance.

Methods
Two independent sample sets: 225 patients from Malmö University Hospital and 100 patients from Skåne University Hospital were used for training, cross-validation and refinement of OncoMasTR. RNA extracted from 225 archived tissues was analysed by RT-qPCR to measure the expression levels of the MTRs. Statistical models of all possible combinations of MTRs were trained and cross-validated (1,000 times x 2-fold) using the first set of 225 samples. Statistical models with the best cross-validated performance were further evaluated on RT-qPCR data from the second independent set of 100 samples. Robustness of the data was verified by assessing the reproducibility of OncoMasTR across 6 days, using 6 unique kit lots, conducted by 4 operators on 3 RT-qPCR instruments.

Results
In the first training set of 225 patients, OncoMasTR classified up to 72% of LN0 patients and 58% of LN0-3 patients as low risk, with ≤5.0% DM within each group. When incorporating CPI, its prognostic performance further improved to a c (concordance) index > 0.8. Results showed that the OncoMasTR molecular score and CPI add statistically significant prognostic value to each other. In the independent verification set, all patients with DM were correctly classified as high risk (p<0.01). In relation to reproducibility, the OncoMasTR test displayed robust performance; the molecular score coefficient of variation was 2.6% across days, kit lots, operators and instruments. Individual MTR assays demonstrated linearity over >2000-fold RNA input range and PCR efficiencies ranged from 92% to 101%.

Conclusions
OncoMasTR development and verification results show analytical robustness and clinically accurate risk stratification. Furthermore, OncoMasTR's binary classification of risk avoids an ambiguous intermediate risk classification and has potential to provide clinicians with useful, actionable information to support treatment decisions. The OncoMasTR test is now ready for large-scale clinical validation.
Margins in breast conserving surgery after neoadjuvant therapy

Jungeun Choi¹, Mehra Golshan¹, Jiani Hu², Haley Cecile Gagnon², Stephen Densantis², Bill Barry² and Tari King¹. ¹Brigham and Women’s Hospital/Dana Farber Cancer Institute, Boston, MA and ²Dana Farber Cancer Institute, Boston, MA.

Background: The margin consensus guideline for patients undergoing primary breast conserving surgery (BCS) and whole breast radiotherapy defines a negative margin as no tumor on ink and concludes that wider margins do not improve local recurrence (LR) rates. There are few studies examining BCS margin width after neoadjuvant chemotherapy (NAC). We sought to determine the impact of margin width on LR and survival rates after NAC and BCS. Methods: Institutional database were reviewed to identify patients with stage I-III breast cancer treated with NAC and BCS from 2002-2014. Patients with inflammatory breast cancer were excluded. Chart review was performed to collect detailed patient and treatment factors. Margins widths were collected as reported and grouped as: positive (ink on tumor), close (<2mm), and negative (>2mm), for the purposes of this analysis. Cox regression was used to determine the relationship between margin width and local recurrence, disease free survival (DFS) and overall survival (OS). Result: 395 patients underwent NAC followed by BCS during the study period. The result was same as below.

<table>
<thead>
<tr>
<th>Patients, n (%)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis (yrs)</td>
<td>51 [22;79]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial tumor size (cm)</td>
<td>3.0 [0.6;11.0]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical node status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- negative</td>
<td>207 (52.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- positive</td>
<td>188 (47.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>subtype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- HR-/Her2-</td>
<td>148 (37.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- HR-/Her2+</td>
<td>48 (12.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- HR+/Her2-</td>
<td>124 (31.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- HR+/Her2+</td>
<td>72 (18.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- unclassified</td>
<td>3 (0.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pCR* status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- pCR</td>
<td>97 (24.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- non pCR</td>
<td>295 (74.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- unclassified</td>
<td>3 (0.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final margin state</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- no residual tumor in breast</td>
<td>108 (27.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- positive</td>
<td>8 (2.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- close (≤2)</td>
<td>99 (25.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- negative (&gt;2)</td>
<td>180 (45.6%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*pCR was defined as no invasive or in situ disease in breast and no tumor in axillary node

Median patient age was 51 yrs (range 22-79); median tumor size at presentation was 3.0 cm (range 0.6-11.0) and 188 (47.6%) patients (pts) presented with clinically node positive disease. Breast cancer subtypes included 148 (37.5%) pts with HR-/Her2-, 48 (12.2%) pts with HR-/Her2+, 124 (31.4%) pts with HR+/Her2- and 72 (18.2%) pts with HR+/Her2+, disease. Among all patients
the pCR rate, defined as no invasive or in situ disease, in the breast was 27.3% (108/395) and the pCR rate in the breast and axillary nodes was 24.6% (97/395). Final margin status included 8 (2.0%) pts with positive margins, 99 (25.1%) with close <2mm and 180 (45.6%) with negative (>2mm) margins. Among the patients with "positive margins"; all were noted to be posterior or anterior and the surgeon noted that reexcision was not possible. At a median follow-up of 53.0 months the LR rate was 2.8% and DFS was 87.4%. On cox regression, HR positive subtype (p=0.048), pCR (p=0.035), and pathologic negative node (p<0.001) were correlated with favorable DFS and pathologic negative node (p<0.001) was correlated with favorable OS. There was no difference in LR rate, DFS or OS between 'close/positive margin' and '>2mm margin groups. **Conclusion:** In this cohort of patients treated with NAC followed by BCS, LR rates were very low and there was no difference in DFS between patients with margins < 2mm or > 2mm. Further studies are needed to confirm the effect of margin width in the NAC setting.
**Title:** Prognostic associations of plasma hepcidin in early breast cancer (BC)

Katarzyna J Jerzak1, Ana Elisa Lohmann2, Marguerite Ennis2, Elizabeta Nemeth3, Tomas Ganz3 and Pamela J Goodwin2.
1Sunnybrook Odette Cancer Centre, Toronto, ON, Canada; 2Mount Sinai Hospital, Toronto, ON, Canada and 3University of California, Los Angeles, Los Angeles, CA.

**Body:** Background: Intra-tumor RNA expression of hepcidin has been linked to adverse metastasis-free survival in women with early BC, but the prognostic implications of this inflammatory marker and iron-regulating peptide are unknown.

**Methods:** Using an ELISA assay, we measured plasma hepcidin in the banked blood of 518 women who were recruited from 1989-1996 for a prospective cohort study regarding diet and lifestyle factors in BC. Blood had been obtained 4-12 weeks post-operatively and prior to treatment with radiation, chemotherapy or hormonal therapy. Women ages 18 to 75 with T1-3, N0-1, M0 BC who underwent surgery and axillary dissection were included; those with metabolic disorders were excluded. Tumor size, grade and ER/PR expression were abstracted from pathology reports; HER2 status was unknown. Median follow-up was 12.1 years (range, 0.2 to 17 years).

Univariable Cox regression models were used to determine the association between hepcidin and i) time to distant BC recurrence (primary outcome), and ii) time to death due to any cause. Multivariable Cox proportional hazards models were adjusted for age (continuous), T stage (T2, T3, Tx vs T1), tumor grade (3 vs 2 or 1), N stage (node positive vs negative), ER/PR expression (both ER and PR negative vs either positive) a-priori. Associations between hepcidin and CRP, IL6, insulin, cholesterol, glucose, vitamin D, total iron, transferrin, and soluble transferrin receptor; sTfR were explored (Pearson's coefficients).

**Results:** Hepcidin ranged from 4.70-190.70 ng/L (median 16.25; IQR 16.40 ng/L). To ensure normal distribution, a transformed \[-1/sqrt (x)\] hepcidin variable was used for prognostic analyses. Average age was 50.3±9.7 years. 16% were obese [body mass index (BMI) >30kg/m^2], 30% (n=156) were node positive, 35% (n=181) had grade 3 tumors and 71% (n=370) had ER and/or PR positive tumors. 77% underwent a lumpectomy, 73% (n=380) received adjuvant radiotherapy and 39% (n=203) received adjuvant chemotherapy.

Plasma hepcidin was not univariably associated with either time to distant BC recurrence (HR for 75th percentile versus 25th 1.20; 95%CI 0.79-1.32) or time to death due to any cause (HR 1.23; 95%CI 0.95-1.59) in the overall cohort; multivariable results were similar. In pre-planned analyses, the prognostic association of hepcidin differed by BMI (≤30 vs >30 kg/m^2; interaction p-values <0.05): among obese women, higher hepcidin was significantly associated with a shorter time to distant BC recurrence in both univariable (HR 1.81; 95%CI 1.06–3.10) and multivariable (HR 1.84; 95%CI 1.04–3.25) models. Higher hepcidin was associated with shorter time to death due to any cause in a univariable model (HR 1.91; 95%CI 1.13–3.22) but not in a multivariable analysis. There was a moderate association between hepcidin and total iron (r=0.35), transferrin (r=0.43) and sTfR (r=-0.39); associations with IL6, CRP and metabolic factors were very weak (r<0.2).

**Conclusion:** Higher plasma hepcidin was independently associated with a shorter time to distant BC recurrence in obese women but not in the overall cohort. Further investigation of hepcidin and mechanisms linking it to adverse BC outcomes is warranted.
**2017 San Antonio Breast Cancer Symposium**

**Publication Number:** P3-08-09

**Title:** Impact of gene-expression profiling in patients with early breast cancer when applied outside the guideline directed indication area

Kay Schreuder¹², Anne Kuijer³, Emiel JTh Rutgers⁴, Carolien H Smorenburg⁴, Thijs Van Dalen⁵ and Sabine Siesling¹².

¹Netherlands Comprehensive Cancer Organisation (IKNL), Utrecht, Netherlands; ²University of Twente, Enschede, Netherlands; ³Diakonessenhuis Utrecht, Utrecht and ⁴Antoni van Leeuwenhoek Hospital – Netherlands Cancer Institute, Amsterdam, Netherlands.

**Body: Purpose**

In Dutch guidelines gene expression profiles (GEP) are indicated in estrogen receptor positive early breast cancer patients in whom benefit of chemotherapy (CT) is controversial based on traditional prognostic factors alone. Aim of the current study is to assess the use and impact of GEP on administration of adjuvant CT in breast cancer patients who have according to national guidelines a clear indication to either use or withhold adjuvant chemotherapy (clinical high or low risk).

**Methods**

Clinical low- and high risk patients, according to Dutch breast cancer guidelines, diagnosed between 2011-2014 were selected from the Netherlands Cancer Registry (NCR). Influence of GEP use and GEP test result on CT administration was assessed with logistic regression.

**Results**

Overall, 26,425 patients were identified; 4.8% of patients with clinical low-risk (444/ 9,354), 7.5% of the patients with a clinical high-risk (1,281/ 17,071) received a GEP. GEP use was associated with a significantly increased odds of CT administration in clinical low-risk patients (OR=2.12 95%CI: 1.44-3.11). In clinical high-risk patients GEP use was associated with a decreased frequency of CT administration (OR=0.55, 95%CI: 0.48-0.63). Adherence to the GEP result was higher in clinical high-risk patients with a discordant GEP result as compared to clinical low-risk patients with a discordant GEP result: 71.7% vs. 52.2%, respectively.

**Conclusion**

GEP is frequently used outside the indicated area and significantly influenced the administration of adjuvant CT, although adherence to the test-result was limited.
Title: Development and validation of a broad-based second generation multi marker “Morphometric IHC” test for optimal treatment planning of stage 1 and 2 breast cancer patients in low resource settings

Somashekhar SP\textsuperscript{1}, Manjiri M Bakre\textsuperscript{2}, Charusheila Ramkumar\textsuperscript{2}, Chetana Basavaraj\textsuperscript{2}, Arunkumar Attuluri\textsuperscript{2}, Lekshmi Madhav\textsuperscript{2}, Chandra Prakash\textsuperscript{2}, Nirupama Naidu\textsuperscript{2} and Sukriti Malpani\textsuperscript{2}. \textsuperscript{1}Manipal Comprehensive Cancer Center, Manipal Hospital, Bangalore, Karnataka, India and \textsuperscript{2}OncoStem Diagnostics Pvt Ltd., Bangalore, Karnataka, India.

Body: Aims:
Assessment of 'risk of recurrence' in ER+ breast cancer patients based on clinical parameters and existing hormone receptor signaling pathway and/or proliferation based biomarkers is insufficient, leading to treatment of majority of patients with chemotherapy. First generation risk identification tests like OncotypeDx and Mammaprint are not impactful in India and SE Asia as are largely prognostic with limited chemotherapy-predictivity and are prohibitively expensive. A cost-effective 'predictive' test which will accurately estimate the 'risk of recurrence' for a 'broader' (node - & +) set of breast cancer patients in low resource settings is urgently required.

Materials and Methods:
Using a retrospective training cohort of 300 node– and node+ patients, we developed 'CanAssist-Breast'- a Morphometric Immunohistochemistry based test comprising 5 biomarkers plus three clinical parameters (Tumor size, grade and node status) to arrive at 'CanAssist-Breast Score'. The risk stratification model was developed using cutting edge support vector based machine learning technology. CanAssist-Breast Score stratifies patients into an all actionable 'low or high' risk for recurrence, with no intermediate zone. CanAssist-Breast biomarkers include cancer stem cell markers, Cadherins, and ATP transporter proteins - all critical players in the various steps of chemotherapy resistance leading to metastasis.

Results:
We validated CanAssist-Breast in accordance with EGAPP recommendations which require that prognostic tests be validated both analytically and clinically prior to being utilized in patients. Analytical validation experiments were performed to assess 'variation' in the outcome prediction due to critical IHC variables. We tested inter-pathologists, sample, operator and laboratory site variation and found high concordance in the outcome predictions across all variables, confirming the robustness and reproducibility of the test.

Extended clinical validation on 1000+ pre and post-menopausal cases shows NPV of 95%. The majority of patients in 'low risk' had Stage 2, Grade 2/3 disease over Stage 1, Grade 1 disease, demonstrating that CanAssist-Breast reclassifies patients who would be considered high risk clinically.

In a head-to-head pilot study of 100 patients with Oncotype Dx, CanAssist-Breast test had about 80% concordance with Oncotype in the 'low risk' category. Importantly, CanAssist-Breast correctly stratified few recurred cases as 'high risk' which were called 'low risk' by Oncotype Dx and thus were not treated with chemotherapy.

Conclusion:
In conclusion, we have developed a robust, accurate and low-cost prognostic test to predict risk of recurrence and enable optimal treatment planning in patients with early stage Breast Cancer.
2017 San Antonio Breast Cancer Symposium

Publication Number: P3-08-11

Title: The prognostic impact of retinoic acid-induced 2 (RAI2) expression in ERα-positive breast cancer patients

Sayaka Nishikawa1, Naoto Kondo1, Yumi Endo1, Yukari Hato1, Tomoka Hisada1, Mayumi Nishimoto1, Yu Dong1, Katsuhiro Okuda2, Hiroyuki Kato3, Satoru Takahashi3, Ryoichi Nakanishi2 and Tatsuya Toyama1. 1Nagoya City University Graduate School of Medical Sciences, Nagoya, Aichi, Japan; 2Immunology and Surgery, Nagoya City University Graduate School of Medical Sciences, Nagoya, Aichi, Japan and 3Nagoya City University Graduate School of Medical Sciences, Nagoya, Aichi, Japan.

Body: Background: Breast cancer cells disseminate to the bone marrow and form bone metastases in a large majority of late-stage patients. Retinoic Acid-Induced 2 (RAI2) was reported as a putative suppressor of early hematogenous dissemination of tumor cells to the bone marrow in breast cancer, particularly in estrogen receptor α (ERα)-positive breast cancer. Here, we investigated mRNA expression of RAI-2 in breast cancer patients during long-term follow-up.

Materials and methods: A total of 451 invasive breast cancer tissues was available for analysis of RAI2 mRNA using a TaqMan PCR system. We also sought correlations between clinicopathological factors and levels of RAI2 expression in these samples. The expression of markers associated with tumor-initiating capacity, such as SNAI1, SNAI2 and VIM was also analyzed. The median follow-up period was 9.0 years. Survival curves were analyzed using the Kaplan-Meier method. Cox proportional hazards regression analysis was used for univariate and multivariate analyses of prognostic values.

Results: We found positive correlations between low expression of RAI2 mRNA and shorter disease-free survival and overall survival in breast cancer patients (P=0.003, P<0.0001, respectively), which was limited to ERα-positive patients (P=0.04, P=0.0009, respectively), and not seen in ERα-negative patients (P=0.52, P=0.27, respectively). Low RAI2 mRNA levels were positively correlated with high grade, ERα-negativity and PgR negativity. Multivariate analysis indicated that low level RAI2 mRNA expression was an independent factor for survival both overall in breast cancer and in ERα-positive breast cancer patients

Multivariate analysis (ERα-positive breast cancer patients)

<table>
<thead>
<tr>
<th></th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Multivariate</td>
</tr>
<tr>
<td></td>
<td>patients  p value</td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
</tr>
<tr>
<td>≤2cm</td>
<td>139</td>
</tr>
<tr>
<td>&gt;2cm</td>
<td>207</td>
</tr>
<tr>
<td>Node status</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>176</td>
</tr>
<tr>
<td>Positive</td>
<td>144</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>124</td>
</tr>
<tr>
<td>2•3</td>
<td>218</td>
</tr>
<tr>
<td>RAI2 mRNA expression</td>
<td></td>
</tr>
<tr>
<td>high</td>
<td>140</td>
</tr>
<tr>
<td>middle•low</td>
<td>206</td>
</tr>
</tbody>
</table>

Conclusion: We show that low expression of RAI2 is an independent factor predictive of a poor prognosis in ERα-positive breast cancer patients. RAI2 could be a promising candidate biomarker and therapeutic target in ERα-positive breast cancer to prevent dissemination to the bone marrow.
Prognostic relevance of neutrophil-to-lymphocyte ratio (NLR) in young women with breast cancer (BC)

Katarzyna J Jerzak¹, Siqi Zhu¹, Sharon Nofech-Mozes¹, Gregory Pond² and Ellen Warner¹. ¹Sunnybrook Odette Cancer Centre, Toronto, ON, Canada and ²McMaster University, Hamilton, ON, Canada.

Body: Background: Many studies suggest that a high NLR prior to systemic treatment is an adverse prognostic marker for BC but its role in women ≤ age 40 in general, and in those with pregnancy associated BC (PABC) in particular is unknown. We investigated whether the NLR is independently prognostic for recurrence-free survival (RFS) in a prospective database of young BC patients.

Methods: A prospective database of women ≤40 years of age diagnosed with BC from 02-2008 to 01-2015 was analyzed. Data regarding age, stage at diagnosis, pathology, treatment and clinical outcomes were available; the NLR was abstracted from the patients’ medical record retrospectively with all values obtained post-diagnosis and prior to systemic therapy. The Kaplan-Meier method was used to estimate time-to-event outcomes, with a primary outcome of RFS. Insufficient events had occurred to analyze overall survival. Univariable Cox proportional hazards regression models were used to evaluate factors that were potentially prognostic for RFS; a subsequent multivariable Cox proportional hazards model adjusted for nodal involvement, PABC status and tumor size. All tests were two-sided and statistical significance was defined as a p-value ≤0.05.

Results: Of 233 women in the database, 208 had a NLR and outcomes available for analysis. The mean age of patients was 35.1 and 24% (n=66/233) had PABC (BC diagnosed during pregnancy or ≤ 24 months postpartum); the median size of their tumors was 2.8 cm (range 0.1 cm – 19.0 cm) and 48% (n=102/211) were node positive. The majority of women had hormone receptor positive (92%; n=206/223) and HER2 negative 75% (n=160/212) disease, 50% (n=112/223) were treated with breast conserving surgery, 61% (n=142/233) received adjuvant radiotherapy and 83% (n=184/223) received adjuvant chemotherapy. With a median follow-up of 41 months, 16 patients (7%) experienced a local recurrence and 25 (11%) had distant recurrent disease. A higher NLR was prognostic for an adverse RFS in both uni- and multi-variable models (Table). The neutrophil count, individually, was also prognostic for adverse RFS but the lymphocyte count was not. The findings for patients with PABC and non-PABC were similar (interaction tests >0.05).

Prognostic association between NLR and RFS

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number of patients (total N=223)</th>
<th>Univariable HR (95% CI)</th>
<th>P value</th>
<th>Multivariable HR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size ( /cm)</td>
<td>198</td>
<td>1.1 (0.9 - 1.2)</td>
<td>0.26</td>
<td>1.0 (0.5 - 1.9)</td>
<td>0.99</td>
</tr>
<tr>
<td>Node positive (yes vs no)</td>
<td>204</td>
<td>2.1 (0.9 - 4.8)</td>
<td>0.091</td>
<td>2.6 (1.0 - 6.7)</td>
<td>0.050</td>
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<tr>
<td>PABC (yes vs no)</td>
<td>223</td>
<td>2.2 (1.0 - 4.8)</td>
<td>0.048</td>
<td>1.8 (0.8 - 4.3)</td>
<td>0.17</td>
</tr>
<tr>
<td>Log[NLR] (/unit)</td>
<td>208</td>
<td>2.6 (1.3 - 5.0)</td>
<td>0.006</td>
<td>2.5 (1.2 - 5.0)</td>
<td>0.006</td>
</tr>
<tr>
<td>Log[neutrophils] (/unit)</td>
<td>208</td>
<td>2.9 (1.2 - 7.3)</td>
<td>0.023</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Log[lymphocytes] (/unit)</td>
<td>208</td>
<td>0.54 (0.16 - 1.8)</td>
<td>0.31</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Conclusions: A higher NLR is prognostic for adverse RFS in our cohort of women ≤40 years of age with BC, including those with PABC. In our study, a higher neutrophil count drives the prognostic effects of NLR, suggesting an inflammatory state. If our results are confirmed in larger data sets, these findings may warrant the investigation of anti-inflammatory agents in the treatment of young women with BC. As BC in this age group has a worse prognosis than BC in older women, the potential benefit of anti-inflammatory agents may be easier to observe in this population.
Title: Serum thymidine kinase activity is an independent prognostic factor for progression-free and overall survival in women with metastatic breast cancer

Anna-Maria Larsson¹,², Sara Jansson¹, Pär-Ola Bendahl¹, Sara Baker¹, Mattias Bergqvist³, Kristina Aaltonen¹ and Lisa Rydén⁴,⁵. ¹Lund University, Lund, Sweden; ²Skåne University Hospital, Lund, Sweden; ³Biovica International AB, Uppsala, Sweden; ⁴Lund University, Lund, Sweden and ⁵Skåne University Hospital, Malmö, Sweden.

Body: Introduction:
Although prognosis and treatment of metastatic breast cancer (MBC) have improved over the last years, there is still an unmet clinical need for more precise prognostic and treatment monitoring tools. Liquid-based markers are preferred since they reflect real-time tumor progression and are not dependent on repeated invasive tissue biopsies. Thymidine kinase 1 (TK1) is an enzyme involved in nucleotide metabolism and has a fundamental role in the DNA synthesis. It can be used as a marker of cell proliferation rate and the TK1 activity has demonstrated correlations to prognosis and usefulness for treatment monitoring in different malignancies. The aim of this study was to determine serum TK1 activity (sTK1) levels measured with the DiviTum assay (Biovica, Sweden), in women with MBC scheduled for 1st line systemic therapy and to evaluate its potential for prediction of outcome and treatment monitoring.

Methods:
142 women with MBC scheduled for 1st line systemic treatment and included in a prospective monitoring trial (CTC-MBC, NCT01322893) were evaluated for sTK1 at baseline (BL) and during treatment at 1, 3 and 6 months. 132 patients had at least one follow-up sample. sTK1 activity levels were measured and correlations to important clinicopathological variables and prognosis (PFS and OS) at BL and during treatment were evaluated.

Results:
The median sTK1 level at BL was 391 u/L (range 10-35520 u/L). When comparing patients with high (above median) versus low (below median) sTK1 levels at BL, high sTK1 levels were found to be associated to worse performance status (p=0.001) and high number of metastatic sites (p=0.03). There was also a statistically significant association between high sTK1 levels and high Ki67 expression in biopsies from metastatic lesions (p=0.038). In univariable analyses high sTK1 levels correlated to worse PFS and OS (HR_{PFS-BL} 2.32, p<0.001; HR_{OS-BL} 2.54, p<0.001) at BL. In multivariable analysis adjusted for clinically used prognostic factors, sTK1 was an independent prognostic factor for PFS and OS (HR_{PFS-BL} 2.4, p<0.001; HR_{OS-BL} 2.0, p=0.01). During treatment, sTK1 was significantly associated with OS from each of the four time points and onwards (BL, 1, 3, 6 months) (HR_{OS-1m} 1.93, p=0.01; HR_{OS-3m} 2.35, p=0.02; HR_{OS-6m} 2.78, p=0.002) in univariable analysis. High sTK1 levels were also associated with impaired PFS (HR_{PFS-1m} 1.48, p=0.06; HR_{PFS-3m} 1.52, p=0.07; HR_{PFS-6m} 2.03, p=0.009) and these associations were significant at BL and 6 months.

Discussion:
sTK1 activity level is an independent prognostic factor for PFS and OS in patients with MBC scheduled for 1st line systemic therapy. During treatment, sTK1 is prognostic for OS evaluated from all time-points up to 6 months. The sTK1 effects observed for PFS are slightly weaker, but still propose potential usefulness for treatment monitoring. Further, sTK1 levels correlate to Ki67 expression in metastatic lesions suggesting that it can be useful as a liquid-based real-time proliferation marker. In conclusion, these results are clinically relevant for prognostication and treatment monitoring in patients with MBC. Future studies of sTK1 are justified to further elucidate in what settings this marker is most useful.
Title: Prediction of distant recurrence in low risk early breast cancer by RT-qPCR based subtyping using MammaTyper®

Mark Laible¹, Kerstin Hartmann¹, Claudia Gürtler¹, Tobias Anzeneder², Stephan Weber³, Thomas Keller³ and Ugur Sahin¹. ¹BioNTech Diagnostics GmbH, Mainz, Germany; ²PATH Biobank, München, Germany and ³ACOMED Statistik, Leipzig, Germany.

Body: Background:
Estrogen receptor (ER/ESR1), progesterone receptor (PR/PGR) and epidermal growth factor receptor 2 (HER2/ERBB2) are routinely assessed by immunohistochemistry (IHC) during workup of breast cancer samples. The routine use of Ki67 (MKI67) IHC assessment in the context of breast cancer subtyping, however, remains controversial, due to poor reproducibility and lack of standardization.

The MammaTyper® test is an in-vitro diagnostic (IVD) test which measures the expression status of the four breast cancer biomarkers ERBB2, ESR1, PGR and MKI67 on the mRNA level via reverse transcription-quantitative PCR (RT-qPCR) and has demonstrated a high degree of reproducibility in the assessment of the four markers.

In this retrospective analysis we assessed the prognostic power of molecular subtyping by MammaTyper® in archived samples from low risk early breast cancers treated with adjuvant endocrine therapy only.

Methods:
ER+/HER2- (according to initial diagnosis) FFPE breast cancer samples from patients treated with adjuvant endocrine therapy only were obtained from 6 different centers. Tumor cellularity was assessed by H&E staining and RNA was extracted from samples with a tumor cell content of ≥20% using a bead-based RNA purification kit (RNXtract®). Total RNA was then used as input for MammaTyper® RT-qPCR. Expression values were classified as positive or negative for each marker based on predefined cutoff values. Tumor subtypes were assigned to each sample based on the combination of binary (pos/neg) single marker expression status according the St Gallen surrogate subtype definition. Distant disease free survival of Luminal A-like samples vs. samples with other subtypes was assessed by Kaplan Meier analysis and Cox regression using SAS version 9.4.

Results:
The final analysis included 319 samples with sufficient tumor cellularity and RNA content for reliable analysis. The rate of distant recurrence in the analyzed set was 8.5%. Median follow up was 7.8 years. The MammaTyper® test called 60% (192) of samples as Luminal A-like (4.7% (9) distant events), 37% (119) as Luminal B-like (HER2 negative) (13.4% (16) distant events), 1.3% (4) as Triple negative (ductal) (25% (1) distant events), 0.6% (2) as “not defined according to St Gallen” (ESR1-/PGR+) (50% (1) distant events) and 0.6% (2) as Luminal B-like (HER2 positive) (no distant events).

When comparing Luminal A-like samples with the samples of the other subtypes in survival analysis, Luminal A-like samples had a significantly better distant disease free survival when assessing samples from patients with pN0 status (278) (p=0.0177, HR=0.344 (95% CI 0.137-0.866), pN1 status (0-3 affected nodes) (314) (p=0.0153, HR=0.374 (95% CI 0.163-0.855) as well as for all samples (p=0.0032, HR=0.319 (95% CI 0.143-0.711).

Conclusion:
Determination of HER2, ER, PR and Ki67 mRNA levels allows molecular subtyping according to the St Gallen surrogate subtype definition. Low risk of distant recurrence could be confirmed for the MammaTyper® Luminal A-like samples suggesting that for this patient group endocrine treatment alone may be sufficient. The high degree of standardization of mRNA measurement may drive the use of the Ki67/MKI67 biomarker in routine breast cancer pathology.
Body: Background: Breast cancer is the most frequent neoplasm in women worldwide and in Mexico, being also the leading cause of cancer death in women. In Mexico, a higher proportion of this tumor subtype (23.1%) has been reported. Patients with TNBC are characterized by aggressive clinical histories and presentation at an early age (in the premenopausal stage), in addition to an increased risk of recurrence and death. However, the main reason for the unfavorable prognosis of these patients lies in the heterogeneity of the disease, as well as the absence of well defined molecular targets that could support the bases of targeted therapy. The heterogeneity in TNBC has been characterized through the knowledge of the biological nature of specific entities within this disease. In 2011 Lehmann et al reported for the first time that TNBC was classified into 6 subgroups. The 6 well-defined subtypes are basal type 1 (BL1), basal type 2 (BL2), immunomodulator (IM), mesenchymal type (M), mesenchymal stem like (MSL), luminal androgen receptor (LAR). In this way the molecular subgroups are sufficiently defined genomically and only little is known clinically of these tumor entities. Therefore, we consider that an important advance for the knowledge of subgroups of TNBC would be the clinico-pathological characterization of these tumors.

Method: To begin with the description of each tumor group, 69 patients were recruited with corroborated diagnosis of TNBC, where all patients had inclusion criteria of 0% positivity to estrogen, progesterone and HER2. To classify our patients we used genomic analysis using the Human Gene 2.0 microarray platform of affymetrix and subsequently 70 samples (a duplicate patient) underwent bioinformatic analysis with the TNBCtype tool developed by Vanderbilt University for access as Web application, whose classification algorithm is based on 3,247 expression profiles of 21 different breast cancer studies from which 6 subgroups of TNBC are identified. We excluded 3 samples because the algorithm indicated that they did not have a TN molecular profile. The remaining 67 samples were analyzed.

Results: The TNBCtype tool classified 67 samples per subtype within the triple-negative type: 10 basal-like (BL1), 11 basal-like (BL2), 16 immunomodulatory, 6 luminal with androgen receptor (LAR), 12 Mesenchymal and 1 mesenchymal stem like. with a significant high of confidence.

Once the patients were classified, overall survival and progression free survival studies were performed per molecular subgroup, as well as univariate and multivariate analyzes with the following variables; Clinical variables: Clinical status, Ki67, CEA (ng / ml), CA 15-3 (U / ml), Neo-adjuvant, Adjuvant, Radiotherapy, Recurrence, Lung metastasis, Liver metastasis and Bone metastasis; Pathologic variables: Histological type, Scarff-Bloom-Richardson grading (SBR), Histological grade, Vascular invasion, EGFR, CK 5/6, ACTIN, RA, P63, CK17 and CK14. Some significant differences were found between groups.

Conclusion: The results obtained so far are the basis for the discrimination of tumor groups with better prognosis versus poor prognosis in triple negative breast cancer.
Title: ER, PR and HER2 biomarkers in UK and Irish clinical breast cancer testing: analysis of results from >168,000 patients

Andrew Dodson¹, Suzanne Parry², Merdol Ibrahim², John Bartlett³, Mitch Dowsett¹ and Keith Miller². ¹Institute for Cancer Research, London, United Kingdom; ²UK NEQAS ICC & ISH, London, United Kingdom and ³ntario Centre for Cancer Research, Toronto, ON, Canada.

Body: AIMS
To describe and analyse >168,000 sets of results from clinical breast biomarker testing carried-out between 2009 and 2016 in the UK and Republic of Ireland, focussing on biological relationships. To present robust confirmatory evidence on known associations and provide new data on previously undescribed or unconfirmed ones.

BACKGROUND
Between Jan-2009 and Jan-2016 the UK National External Quality Assessment Scheme for Immunocytochemistry and In-situ Hybridisation (UK NEQAS ICC&ISH) collected results on clinical breast cancer testing, comprehensively for human epidermal growth factor receptor-2 (HER2) and optionally for estrogen receptor (ER) and progesterone receptor (PR), from most UK and Irish testing centres. Primary objectives were to assess and, where indicated achieve improvements in testing quality. The size and scope of the data-set is unparalleled in the literature and represent a significant reference resource for the breast cancer community.

METHODS
UK NEQAS ICC&ISH created and curated an on-line data-entry system allowing centres to systematically collect their own HER2, ER and PR results from clinical testing; for HER2, immunohistochemical (IHC) and in-situ hybridisation (ISH) data were collected; for ER and PR, IHC results could be entered according to local practise format. Tools and guidance were provided enabling centres to analyse their data for local audit and quality assurance. Clinicopathologic data was also collected, including: patient age at diagnosis, histological tumor type, tumor grade, site/stage (primary, recurrence, metastasis) and sample type (core, excision).

RESULTS
Data are present on 168,793 patients. 173 centres contributed ≥100 entries (96% of total). Median age was 62 years (IQR: 51-72). Tumor type was stated in 42%: 76% were invasive ductal, and 13% invasive lobular carcinoma. Grade was stated in 56%: 15% were Grade 1, 55% Grade 2 and 30% Grade 3. Site/stage was stated in 56%: 92% were primary, 2% recurrent and 6% metastatic. Sample type was stated in 70%: 75% were cores and 25%, excision.

Receptor statuses were available as follows; HER2 (100%): 87% negative, 13% positive; ER (45%): 15% negative, 85% positive; PR (31%): 29% negative, 71% positive.

HER2 data were available as follows; category by IHC: 91%; amplification status by ISH: 15%; HER2 gene/chromosome 17 (CEP17) ratio: 15% (86% of which were IHC 2+); HER2 gene copy number: 7%; CEP17 copy number: 7%.

HER2-positive rate was 24% in ER-negative and 11% in ER-positive cases. HER2-positive status was negatively associated with increasing ER positivity (rho -0.22, P<0.001). 71% of HER2-positive cases were ER-positive and 29% were ER-negative. The HER2 2+ rate was 14% in ER-negative and 21% in ER-positive cases. Considering only IHC 2+ cases, median HER2 copy number was 5.4 in ER-negative and 4.4 in ER-positive disease.

Comprehensive description of significant associations for all parameters will be presented.

CONCLUSION
The unique size and scope of this data-set has allowed confirmation of known associations for HER2, ER and PR with clinicopathologic and biological characteristics in breast cancer to very high confidence levels, and has uncovered previously undescribed relationships in both ER-positive and ER-negative disease.
Title: Vitamin D and breast cancer risk by pathologic subtypes in Spain (MCC-Spain)

Marina Pollán1,2, Virginia Lope1,2, Adela Castelló1,2, Antonio Mena-Bravo6, Pilar Amiano2,4, Nuria Aragonés1,2, Tania Fernández-Villa2,5, Marcela Guevara2,6, Trinidad Dierssen2,7, Guillermo Fernández-Tardón2,8, Gemma Castaño-Vinyals2,9, Rafael Marcos-Grajera2,10, Victor Moreno2,11, Dolores Salas-Trejo12, Marian Díaz-Santos13, Manolos Kogevinas2,9, Beatriz Pérez-Gómez1,2 and Feliciano Priego-Capote3. 1National Center for Epidemiology; Carlos III Institute of Health, Madrid, Spain; 2Consortium for Biomedical Research in Epidemiology & Public Health (CIBERESP), Madrid, Spain; 3University of Córdoba, Córdoba, Spain; 4Public Health Division of Gipuzkoa, BioDonostia Research Institute, Sam Sebastian, Guipuzcoa, Spain; 5University of León, Institute of Biomedicine (IBIOMED), León, Spain; 6Instituto de Salud Pública de Navarra, IdiSNA, Pamplona, Navarra, Spain; 7University of Cantabria—IDIVAL, Santander, Cantabria, Spain; 8IUOPA University of Oviedo, Oviedo, Asturias, Spain; 9ISGlobal, Centre for Research in Environmental Epidemiology (CREAL), Barcelona, Spain; 10Epidemiology Unit & Girona Cancer Registry, Oncology Coordination Plan, Autonomous Government of Catalonia, Girona, Spain; 11IDIBELL-Catalan Institute of Oncology, L’Hospitalet de Llobregat, Barcelona, Spain and 12Consellería de Sanidad Universal y Salud Pública, Generalitat Valenciana, Fundación para el Fomento de la Investigación Sanitaria y Biomédica de la Comunidad Valenciana (FISABIO), Valencia, Spain.

Body: Introduction: Vitamin D (VD) is involved in proliferation, apoptosis, differentiation, invasion and metastasis. Epidemiological studies show a clear protective effect of VD for colorectal cancer, while the evidence for breast cancer (BC) is less conclusive. Up to 50% of the population worldwide may have insufficient levels of 25-OH-vitamin D (25OHVD), the biomarker reflecting VD status. Our goal was to study the association between serum 25OHVD and BC risk by pathologic subtype in a subsample of BC cases/controls from a large case-control study in Spain.

Methods: MCC-Spain is a population-based multicase-control study on environmental and genetic factors (http://www.mccspain.org/). Here, we selected 558 BC cases who donated blood samples before starting chemotherapy, radiotherapy or hormonotherapy and 558 controls were frequently matched -by region, age and body mass index (BMI). 25OHVD was estimated by chromatograph-mass spectrometer in a sample of 200 µL serum. The effect (OR) of 25OHVD was estimated from mixed logistic regression models (random term = region), adjusted for age, BMI, menopausal status, reproductive factors, family history of BC, previous biopsies, hypercholesterolemia, reproductive factors, family history of BC, previous biopsies, hypercholesterolemia, hormonal treatment, skin color, tobacco, physical activity and season of sampling. We used multinomial models -with the same co-factors- to estimate the association according to BC pathologic subtypes: a) luminal: ER+ or PR+ with HER2-, b) HER2+, and c) triple negative (TN): ER-, PR- and HER2-.

Results: 25OHVD levels were available in 546 cases and 558 controls. Mean 25OHVD was 48.2 nmol/L in controls and 43.6 in cases (P<0.01). Overall, BC risk decreased with increasing 25OHVD levels (OR per 10 nmol/L=0.88; P<0.001), with no differences in pre- and postmenopausal women. While similar ORs were found for luminal and HER2+ tumors (OR per 10 nmol/L= 0.89 and 0.88 respectively), a stronger effect was observed for TN (OR=0.64, P-heterogeneity: 0.039). No differences were observed according to TNM stage (P-heterogeneity=0.706). Among BC cases, similar 25OHVD levels were observed in cases sampled in weeks 1&2 and weeks 3&4 after diagnosis, but 25OHVD level was lower among cases sampled > 1 month after diagnosis. A sensitivity analysis including only BC cases sampled in the first month showed a slightly lower effect (OR per 10 nmol/L=0.92; P=0.034), similar to the effect reported in meta-analyses of cohort studies. This analysis confirmed the stronger association found for TN tumors (OR= 0.53, P=0.001; P-heterogeneity=0.012). Finally the dose-response analysis showed a downward (protective) effect up to concentrations around 90 nmol/L, from which the risk increased. However, only 3% of women had levels over 90 nmol/L.

Conclusions: To our knowledge, this is the 1st study providing information on the effect of VD by BC subtypes. Our results show a clear protective effect, particularly against TN tumors.

Funding: Spain’s Health Research Fund (Fondo de Investigación Sanitaria - FIS PI12/00488); Ministry of Health (EC11-273).
Title: Risk stratification using clinical risk factors and genetic variants in a personalized screening trial

Yiwey Shieh\(^1\), Elad Ziv\(^1\), Martin Eklund\(^2\), Leah Sabacan\(^1\), Roxanna Firouzan\(^1\), Lisa Madlensky\(^3\), Hoda Anton-Culver\(^4\), Alexander Borowsky\(^5\), Andrea LaCroix\(^3\), Arash Naeim\(^6\), Barbara Parker\(^3\), Laura van't Veer\(^1\), Laura Esserman\(^1\), Jeffrey Tice\(^1\) and WISDOM Study and Athena Network Investigators\(^7\). \(^1\)University of California San Francisco, San Francisco, CA; \(^2\)Karolinska Institutet, Stockholm, Sweden; \(^3\)University of California San Diego, San Diego, CA; \(^4\)University of California Irvine, Irvine, CA; \(^5\)University of California Davis, Sacramento, CA; \(^6\)University of California Los Angeles, Los Angeles, CA and \(^7\)WISDOM Study and Athena Network Investigators.

Body: Introduction: Tailoring breast cancer screening according to individual risk may represent an improvement over the current practice of age-based screening. WISDOM (Women Informed to Screen Depending on Measures of Risk) is an ongoing randomized trial comparing the safety, efficacy, cost, and patient acceptability of personalized versus annual screening. Women in the personalized arm receive screening recommendations based on sequencing of 9 genes associated with hereditary breast cancer and a 5-year risk estimate from the Breast Cancer Surveillance Consortium (BCSC) risk model modified by a polygenic risk score (PRS) comprised of 75 single nucleotide polymorphisms. WISDOM represents the first-ever use of a PRS to prospectively modify risk estimates and allows comparison of risk model performance in a population-based setting. Thus, we evaluated the risk estimates generated by: 1) the Breast Cancer Risk Assessment Tool (BCRAT) based on the Gail model, 2) the BCSC model, and 3) the BCSC model modified by the PRS (BCSC-PRS).

Methods: We analyzed participants in the personalized screening arm of the WISDOM Study (NCT02620852). The trial opened in October 2016 and is enrolling participants aged 40-74 years. Participants’ self-reported demographic and risk factor information were collected through an online portal. Genotyping of participants in the personalized arm was done using a custom panel from Color Genomics. 5-year risk estimates were generated using the BCRAT (2011 version), BCSC, and BCSC-PRS models. In the latter, the PRS was used as a Bayesian likelihood ratio to modify the BCSC 5-year risk estimate. We compared the distributions of BCRAT, BCSC, and BCSC-PRS risk estimates around a low-risk (<1%) and moderately high-risk (≥3%) threshold using a paired statistical test (McNemar).

Results: To date, WISDOM has enrolled 2,065 participants, of whom 1,157 are in the personalized arm and 830 have completed risk assessment. The median age was 57 years (interquartile range, IQR 49-64). 83% were Caucasian, 2% African-American, and 7% Asian. 8% self-reported as Hispanic. The median 5-year risk was 1.7% (IQR 1.1-2.3%) using the BCRAT, 1.6% (IQR 1.1-2.3%) using the BCSC model, and 1.5% (IQR 0.9-2.7%) using the BCSC-PRS model. The BCSC-PRS model classified more women into the low (<1%) and moderately high-risk (≥3%) risk categories compared with the BCRAT (p < 0.001) and BCSC model (p < 0.001), Table.

<table>
<thead>
<tr>
<th></th>
<th>&lt;1%</th>
<th>1-3%</th>
<th>≥3%</th>
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<tr>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td><strong>Gail</strong></td>
<td>161 (19)</td>
<td>556 (67)</td>
<td>113 (14)</td>
</tr>
<tr>
<td><strong>BCSC</strong></td>
<td>159 (19)</td>
<td>568 (68)</td>
<td>103 (12)</td>
</tr>
<tr>
<td><strong>BCSC-PRS</strong></td>
<td>275 (33)</td>
<td>379 (46)</td>
<td>176 (21)</td>
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Discussion: Adding a PRS to the BCSC model categorized significantly more women below the low-risk threshold and above the moderately high-risk threshold compared with the BCSC model and BCRAT. Furthermore, the BCSC and BCRAT generated similar distributions of risk estimates. Follow-up with incident breast cancer data is needed to determine whether the reclassification provided by the PRS improves risk stratification and clinical outcomes. However, our preliminary findings suggest that incorporating genetic variants into a validated clinical model is feasible and could enhance risk prediction.
Title: A comprehensive tool for measuring mammographic density changes over time

Mikael Eriksson¹, Jingmei Li¹, Karin Leifland², Kamila Czene¹ and Per Hall¹. ¹Karolinska Institutet, Stockholm, Sweden and ²South General Hospital, Stockholm, Sweden.

Body: Background. Mammographic density is a marker of breast cancer risk and diagnostics accuracy. A change in mammographic density over time is potentially a stronger predictor of breast cancer and a strong proxy for response to endocrine treatment. We developed the STRATUS software to enable automated measurements of density changes over time in research studies and clinical follow-up.

Method. Raw and processed images from the same mammogram were sampled from ~40,000 healthy women. Mammographic density was measured on raw images, using FDA approved software (iCAD), and used as a template for machine learning of STRATUS to measure density on processed images. A similar two-step design was used to train density measures in analogue images. Relative risks of density were estimated in three case-control studies. An alignment protocol was developed on 11,409 women to reduce non-biological variability in density change. The protocol was evaluated in 15,256 women having two regular mammography screens. Difference and variance of density were compared before and after image alignment.

Results. The relative risk of percent mammographic density was 1.6 (95% confidence interval CI 1.3-1.8) per standard deviation. The discrimination was AUC 0.62 (CI 0.60-0.64). Type of image did not influence the risk associations. Alignment decreased the non-biological variability in density change from SD 2.4 to SD 1.8 cm², p<0.001.

Conclusion. STRATUS density measures showed same risk estimates regardless of type of image used. The alignment protocol identified a true density change over time. STRATUS has the potential to become a useful tool for research and clinical follow up.
Body: **Introduction:** The 21-gene recurrence score (RS) assay categorizes hormone receptor positive, node negative breast cancers (BC) into 3 risk groups for recurrence. We previously showed that the AAMC Model, using only standard pathology data, accurately does the same. This study compares the recurrence rate of the AAMC Model's risk groups to RS-based risk groups. A 2-step approach then is used, in which the AAMC model is applied first, and the RS assay is used only for AAMC intermediate risk cases. AAMC intermediate cases were reclassified by RS into low or high risk groups.

**Methods:** From a prospective registry of newly diagnosed BC, we selected invasive, hormone receptor positive, HER2 negative, lymph node negative cases from 2005 to 2015 tested with RS assay. Five-year Kaplan-Meier distant recurrence rates were calculated for each risk category.

**Results:** 1268 cases were included. Five-year recurrence rates were similar between the AAMC Model's low risk group and RS<18 low risk group, as well as between the AAMC Model's high risk group and the RS>30 high risk group. Applying the RS assay to the 715 cases in the AAMC Model's intermediate group resulted in re-classifying 417 (58%) as low risk and 41 (6%) as high risk. Using RS alone, 33% of cases were intermediate risk (n=424), whereas in the 2-step approach 20% were intermediate risk (n=257). For the 2-step approach, the 5-year distant recurrence rate was 3.3% for the low risk group (n=740) and 24.4% for the high risk group (n=271).

**Conclusions:** Five-year recurrence rates in the AAMC Model's low and high risk groups were similar to those in RS-based risk groups. The 2-step approach, with RS used only for AAMC intermediate cases, resulted in larger low and high risk groups with equivalent prognostic accuracy, compared to use of the RS assay alone. The 2-step approach reliably identifies a large number of patients unlikely to benefit from 21 gene assay and provides substantial cost savings.

### Kaplan-Meier Calculated 5-year Distant Recurrences Rates for 4 Models: 1268 Patients

<table>
<thead>
<tr>
<th></th>
<th>Oncotype DX</th>
<th>TAILORx</th>
<th>AAMC Model</th>
<th>2 Step Model with OncotypeDX for AAMC Intermediates</th>
</tr>
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<tbody>
<tr>
<td><strong>Low Risk</strong></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>RS &lt; 18 (n=703)</td>
<td>RS &lt; 11 (n=250)</td>
<td>Grade 1 and PR ≥ 1% (n=323)</td>
<td>AAMC Low or AAMC intermediate/RS &lt;18 (n=740)</td>
<td></td>
</tr>
<tr>
<td>3.4% (95% CI 1.6 – 5.1%, nf=17)</td>
<td>4.0% (95% CI 0.8 – 7.2%, nf=8)</td>
<td>2.7% (95% CI 0.0 – 5.4%, nf=5)</td>
<td>3.3% (95% CI 1.4 – 5.2%, nf=16)</td>
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<tr>
<td><strong>Intermediate Risk</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>RS 18 - 30 (n=424)</td>
<td>RS 11 - 25 (n=787)</td>
<td>Not meeting AAMC definition for low or high risk (n=715)</td>
<td>AAMC Intermediate and RS 18-30 (n=257)</td>
<td></td>
</tr>
<tr>
<td>15.2% (95% CI 10.3 – 20.1%, nf=38)</td>
<td>7.3% (95% CI 4.7 – 9.9%, nf=35)</td>
<td>8.4% (95% CI 5.4 – 11.3%, nf=36)</td>
<td>12.0% (95% CI 5.8 – 18.1%, nf=15)</td>
<td></td>
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<tr>
<td><strong>High Risk</strong></td>
<td></td>
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<tr>
<td>RS &gt; 30 (n=141)</td>
<td>RS &gt; 25 (n=231)</td>
<td>Grade 3 or ER &lt; 20% (n=230)</td>
<td>AAMC High or AAMC intermediate/RS &gt; 30 (n=271)</td>
<td></td>
</tr>
<tr>
<td>23.0% (95% CI 14.7 – 31.3%, nf=27)</td>
<td>22.9% (95% CI 15.9 – 29.9%, nf=39)</td>
<td>22.8% (95% CI 16.1 – 29.5%, nf=41)</td>
<td>24.4% (95% CI 18.0 – 30.7%, nf=51)</td>
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</tr>
</tbody>
</table>

RS= Recurrence Score, nf=number of recurrences, CI = confidence interval.
2017 San Antonio Breast Cancer Symposium

Publication Number: P3-09-06

Title: Predicting cardiovascular versus cancer mortality in a cohort of breast cancer survivors

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Body: Background: Given improved survival after breast cancer diagnosis for women with non-metastatic disease, many will likely survive their disease and ultimately die from causes other than breast cancer, the most frequent being cardiovascular disease. There are numerous risk prediction models, such as the Framingham risk score, to identify persons who are at high risk for a cardiovascular event or death. However, these models have been developed for use in the general population and have not been validated in any cohorts of cancer survivors, who are at increased risk for competing causes of death. We evaluated commonly used risk models for cardiovascular events on a contemporary cohort of breast cancer survivors, and developed a new risk model to simultaneously predict the likelihood of death from breast cancer or cardiovascular disease (CVD).

Methods: We included all women diagnosed with stage I-III breast cancer between January 1, 2000 and December 31, 2010 in Kaiser Permanente Northern California (KPNC) with follow-up through April 30, 2015. Specifically, we extracted from KPNC clinical and other databases: breast cancer characteristics, cardiovascular risk factors (cholesterol, blood pressure (BP), diabetes, BP lowering medication, smoking status), cardiovascular events, and cause of death. We assessed discrimination for the Framingham, CORE and SCOREOP cardiovascular risk models using the area under the receiver operating characteristic curve (AUC), and calibration by comparing the observed to the expected events. We used a multi-state model based on Cox cause specific hazards (CSH) to jointly model the risk of cardiovascular death and breast cancer death, while accounting for all other causes.

Results: In this population of 20,462 KPNC breast cancer survivors with a median follow-up of 7.5 years, there were 695 cardiovascular and 842 breast cancer deaths. The existing cardiovascular risk models discriminated adequately (AUCs ranging 0.64 – 0.78), though models predicting cardiovascular mortality tended to over-predict, while those predicting non-fatal events tended to under-predict. Models developed to predict in a shorter time frame (<5 years), performed slightly better (E/O ratios of 1.08 and 1.18 for Framingham predicting events in the next 2 and 4 years, respectively). In our multi-state model, many of the traditional cardiovascular risk factors were no longer statistically significant (diabetes, BP) in predicting cardiovascular mortality, while the breast cancer characteristics (grade, tumor size, nodal involvement), as well as a prior history of CVD, were most useful in predicting cause of death. The model performed well, with AUCs of 0.85 (95% CI 0.83, 0.86) for 5-year risk of cardiovascular death and 0.85 (95% CI 0.84, 0.87) for breast cancer death.

Conclusion: If replicated in an independent cohort, our model suggests that breast cancer characteristics can help predict overall mortality as well as cardiovascular death. Given the risk of cardiovascular death in the population of breast cancer survivors, joint modeling of breast and cardiovascular mortality is warranted.
Title: Breast cancer risk assessment in a multiethnic patient population

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Body: The United States Preventive Services Task Force recommends that women who are at increased risk for breast cancer and at low risk for adverse medication effects should be offered risk-reducing medications, such as tamoxifen or raloxifene, by their clinicians. In addition, the National Comprehensive Cancer Network recommends risk counseling for women with a 5-year risk of $\geq 1.7\%$ as calculated by the NCI-developed Breast Cancer Risk Assessment Tool (BCRAT, based on the Gail model) or other risk model. Thus, breast cancer risk assessment is important for the identification of women at "high risk" who should be offered risk counseling and potentially intervention. The Athena Breast Health Network, which has served $>120,000$ breast screening patients across California and the midwest, has integrated breast cancer risk assessment into its clinical breast screening programs. The goal of our study was to characterize breast cancer risk for $>10,000$ mammography patients in the University of California Irvine Athena Breast Health Network, overall and by race/ethnicity, using several different risk models, including the BCRAT, BCSC, and IBIS models. Our cohort was comprised of 47\% non-Hispanic White, 13\% non-Hispanic Asian, 38\% Hispanic, and 2\% women of other race/ethnicities. Using data collected from electronic medical records and self-completed questionnaires, we determined that, as expected, non-Hispanic White and Asian women had higher breast cancer risk scores than Hispanic women for all risk models (5-year risks = 1.51-1.68\% and 1.22-1.40\% vs. 0.95-1.05\%, respectively). In addition, when women were categorized as "increased risk" according to a given risk model if their 5-year risk score was $\geq 1.7\%$, the percentages of women at "increased risk" were higher in White women (26.5–42.2\%) than in Asian (15.8–28.6\%) and Hispanic (6.2–10.7\%) women. However, the correlations between risk models were low to moderate in our cohort, overall (Pearson's $r = 0.47-0.62$) and especially for Asian women (Pearson's $r = 0.29-0.49$). Our results indicate that using only one risk model in a clinical breast cancer risk assessment program to identify "high risk" women would miss a significant proportion of women who would have been considered "high risk" according to another risk model. Conversely, some women who are identified as "high risk" according to one model may not need risk counseling and intervention since they are not considered "high risk" according to two other models. As our cohort expands and incident breast cancers occur, we will be able to determine which risk model or combination of risk models will have the highest discriminatory accuracy for predicting breast cancer risk in women of different race/ethnicities, which will enable our risk assessment programs to have a more targeted approach to risk counseling and intervention.
Title: Overeating and breast cancer risk by pathologic subtypes: EpiGEICAM study

Body: Introduction: It has been reported that overeating may be the greatest avoidable cause of cancer in nonsmokers and obesity increases postmenopausal breast cancer (BC) risk. Calorie restriction reduces BC incidence in experimental animals, but the evidence in humans is more limited. This study analyzes the association between female BC risk and excessive or restricted calorie consumption in Spain.

Methods: EpiGEICAM is a multicenter case-control study including 1017 matched pairs (age & town) of incident BC cases and healthy controls in 14 regions in Spain. Participants filled a structured questionnaire. Average calorie intake (cals) during the 5 years previous to diagnosis (cases) or interview (controls) was estimated using a validated 117-item semiquantitative food-frequency questionnaire. Expected calorie intake (cals_exp) was calculated from a linear regression model taking into account the basal metabolic rate (Sabounchi’s meta-regression) and the amount of physical activity performed by each woman (5 categories). For each woman the prediction interval (99%) of cals_exp was used to consider her calorie intake as “normal” (NCI) (cals inside this interval –the reference group-), “excessive” (ECI) (cals>upper limit of the interval) or “restricted” (RCI) (cals<lower limit of the interval). The association of ECI and RCI with BC, overall and by pathologic subtype (luminal (ER+ and/or PR+ with HER2-), HER2+ and triple negative), was evaluated using conditional and multinomial logistic regression models, adjusted for age and region (multinomial models), education, body mass index (BMI), smoking, age at menarche & at first birth, menopausal status, previous history of benign breast disease, family history of BC, hormonal replacement therapy (HRT), physical activity and two scores reflecting the participant’s adherence to Mediterranean and Western dietary patterns.

Results: After excluding participants with extreme calorie intake, 973 case-control pairs were considered. Average number of calories was higher in BC cases (1990 kcals) than controls (1897 kcals) \(P=0.001\). Women with RCI (cals<80% of the predicted limit) had lower BC risk (OR=0.52, \(P=0.001\), being this effect more marked in premenopausal women (OR=0.36; \(P=0.001\). On the contrary, women with ECI (cals>40% of the predicted limit) showed increased BC risk (OR=1.92; \(P=0.001\) being this effect stronger in postmenopausal women (OR=2.61; \(P=0.001\). By pathologic subtypes, no statistically significant differences were observed, but ECI (over 40%) was strongly associated with HER2+ tumors (OR=2.05, \(P=0.021\). No differences in the effect of ECI or RCI were observed by levels of BMI, tobacco or HRT.

Conclusion: After taking BMI into account, excessive energy intake increases BC risk, while relative caloric restriction seems to have a protective effect. Moderate calorie restriction, in combination with regular physical activity, could be a good strategy for BC prevention.

Funding: Scientific Foundation of the Spanish Association Against Cancer (AECC), SEOM, FECMA, Cerveza y Salud Foundation, FIS CD110/00018
Title: Assessing utility of breast cancer risk assessment tool in comparison to Tyrer-Cuzick model for determination of breast cancer risk and implications for chemoprevention

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Body: Background
Despite findings that the Tyrer-Cuzick (IBIS Breast Cancer Risk Evaluation Tool or TC) model is more predictive of breast cancer risk than the Gail model (NCI maintained Breast Cancer Risk Assessment Tool or BCRAT), BCRAT is commonly clinically used as per the United States Preventive Services Task Force (USPSTF), with a 5-year risk for breast cancer (BC) of greater than 3% on BCRAT, the benefits of preventive medication likely outweigh the risks. We aimed to compare the models, 1: to see if a 10 year risk estimate per the TC model reliably correlated with the 3% 5 year risk per BCRAT, and 2: to analyze the subset of patients with atypical hyperplasia (AH) and lobular carcinoma in situ (LCIS) who are known both to be at high risk for breast cancer and to benefit from chemoprevention. Our hypothesis is that BCRAT has limited utility in risk estimation, and the most comprehensive model for risk estimation and clinical decision making is TC.

Methods
200 women ages 35-64 women followed in benign breast clinic were included. Risk estimations were run using BCRAT, TC version 7 (v7) and TC version 8 (v8). A Pearson's Correlation test was conducted to investigate the relationship between the TC models and the BCRAT model. A p-value < 0.05 was considered statistically significant.

Results
Analysis showed a positive moderate-strength relationship between the TC v7 10-year risk and the 5-year BCRAT risk for this population (R = 0.468, P<0.001) and a positive moderate-strength relationship between the TC v8 10-year risk and the 5-year BCRAT risk (R = 0.550, P<0.001). A TC v7 risk of 8.09% (95% confidence interval (CI): 7.42-8.75) and a TC v8 risk of 8.54% (95% CI: 7.85-9.24) corresponded to a BCRAT risk of 3%. However, much error was present when assessing consistency and correlation between the models.

A total of 36 patients were diagnosed with AH, 2 patients were diagnosed with LCIS and 7 patients were diagnosed with both AH and LCIS. 11 patients who had AH had an estimated 5-year risk per the BCRAT model of <3%. Two of these patients were pre-menopausal and African American and one was pre-menopausal and Hispanic. Of the remaining 8 patients, all were under the age of 60.

Of the 30 patients who had a BCRAT 5-year estimated risk of BC of >3% but no AH or LCIS, 12 had two first degree relatives with breast cancer and 16 had a first-degree relative with BC and at least two benign breast biopsies.

Conclusion
BCRAT is limited and caution is warranted with its use in assessing risk and for counseling around chemoprevention benefit. There is not reliable correlation between the 5 year BCRAT risk estimate and the 10 year TC risk estimate. Chemoprevention should be discussed for patients with AH, LCIS or 2+ first degree relatives with breast cancer. Further, BCRAT may underestimate risk in minority populations and others with AH. For a limited group of patients with moderate risk, dual modeling may be clinically useful in making chemopreventive recommendations.
Body: Introduction: Although breast cancer (BC) is the most frequent tumor in women worldwide, well-established risk factors account for 40%-50% of cases. The influence of environmental factors is not well known. Our objective was to assess the association between residential proximity to industrial facilities included in the European Pollutant Release and Transfer Register and risk of BC, according to different categories of industrial groups and specific pollutants released, in the context of a multicentric population-based case-control study carried out in Spain (MCC-Spain).

Methods: MCC-Spain is a frequency matched, multicase-control study that evaluates environmental and genetic factors associated with the risk of BC and other tumors in 12 regions in Spain (http://www.mccspain.org/). Given the study design, the present work was restricted to small administrative divisions including both BC cases and controls. A total of 452 BC cases and 1511 controls from the original set were included. Using GIS methods, distances were computed from the respective subject’s residences to the 116 industries located in the study areas. We used logistic regression to estimate odds ratios (ORs) and 95% confidence intervals (95%CIs) for categories of distance (from 1 km to 3 km) to industrial facilities, adjusting for age, family history of breast cancer, age at menarche, educational level, body mass index one year before the interview, age at first birth, menopausal status, and previous biopsies.

Results: Excess risk (OR; 95%CI) of breast cancer was detected near industries as a whole (1.30; 1.00-1.69 at 3 km), particularly organic chemical industry (2.12; 1.20-3.76 at 2.5 km), food and beverage sector (1.87; 1.26-2.78 at 3 km), ceramic (4.71; 1.62-13.66 at 1.5 km), surface treatment using organic solvents (2.00; 1.23-3.24 at 3 km), and surface treatment of metals and plastic (1.51; 1.06-2.14 at 3 km). By pollutants, the excess risk (OR; 95%CI) was found in women close to industries releasing pesticides (2.09; 1.14-3.82 at 2 km), and dichloromethane (2.09; 1.28-3.40 at 3 km).

Conclusions: Our results suggest a possible increased risk of BC in women living near certain industrial facilities. These findings support the need for more detailed exposure assessment of certain toxics released by these industries.

Funding: Spain’s Health Research Fund (Fondo de Investigación Sanitaria - FIS PI12/00488) and Scientific Foundation of the Spanish Association Against Cancer (Fundación Científica de la Asociación Española Contra el Cáncer (AECC) – EVP-1178/14)
A genetically underserved community

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Body: It is estimated 5-10% of breast cancer can be attributed to a hereditary predisposition. By knowing a woman’s risk for breast cancer, risk reduction strategies can be discussed. Waco, Texas is an underserved community with the nearest geneticists and genetic counselors 35 miles away. This is a multidisciplinary approach to developing a breast cancer risk assessment and genetic carrier screening program in Waco, Texas.

Program Development The initial pilot was designed as a two-week trial to see what the prevalence of at risk women for breast cancer is in our community. The Breast Center distributed a cancer history questionnaire to all of their patients. The women were scored on the Tyrer-Cuzik Breast Cancer Lifetime Risk Assessment Model and surveyed for family and personal history of cancer. Total patients screened in the Breast Center 619. Of these 63.8% had a personal or family history of cancer and 21.8% meet NCCN 2015 criteria for genetic testing. It was projected a 5.3% mutation carrier status in our population and a 14% high risk breast cancer population (>20% lifetime risk of breast cancer).

Methods As a quality initiative, the Breast Center distributed the screening questionnaire and Tyrer-Cuzik score for each woman. Each questionnaire is assessed by a NP for genetic evaluation per NCCN guidelines Genetic/Familial High-Risk Assessment: Breast and Ovarian v1.2016 and NCCN Genetic/Familial High-Risk Assessment; and Colorectal v.1.2016. Allowing for insurance coverage differences, if the patient meets the above criteria, the patient is offered an appointment with informed consent, and genetic counseling services provided by the NP allowing for testing same day as consultation. This program is ongoing, the results listed began January 4, 2016 and ended December 30, 2016. Total patients seen in Breast Center 11,196. Total patients screened for family history 9,726. Patients with family or personal history was 51%, of that 24% met NCCN genetic testing criteria. Patients tested 316, with 5.7% identified genetic mutations

<table>
<thead>
<tr>
<th>Gene</th>
<th>Number of Patients</th>
<th>Associated risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1</td>
<td>2</td>
<td>Up to 87% breast cancer; 44% ovarian cancer risk</td>
</tr>
<tr>
<td>BRCA2</td>
<td>1</td>
<td>Up to 84% breast cancer; 27% ovarian cancer risk</td>
</tr>
<tr>
<td>MSH6</td>
<td>1</td>
<td>Up to 58% risk of male and 30% female colon cancer, up to 71% endometrial cancer</td>
</tr>
<tr>
<td>CHEK2</td>
<td>3</td>
<td>Up to 48% risk breast cancer; 9.5% colorectal cancer</td>
</tr>
<tr>
<td>PALB2</td>
<td>1</td>
<td>Up to 40% breast cancer risk</td>
</tr>
<tr>
<td>PMS2</td>
<td>1</td>
<td>Up to 20% colorectal cancer risk, up to 15% endometrial cancer risk</td>
</tr>
<tr>
<td>SMAD4</td>
<td>1</td>
<td>Up to 50% colorectal cancer risk, 21% risk of gastric cancer</td>
</tr>
<tr>
<td>MUTYH, monoallelic</td>
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<td>increased risk of colon cancer</td>
</tr>
<tr>
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<td>increased risk ovarian cancer</td>
</tr>
<tr>
<td>NBN</td>
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<td>increased breast cancer</td>
</tr>
</tbody>
</table>

Women with a >20% lifetime risk of breast cancer 14%.

Conclusion The first year of the program, demonstrated our patient population has a 5.7% prevalence of mutation detection. In addition, a 7.2% high risk breast cancer prevalence was identified. It is imperative to bring awareness to underserved communities to reduce the risk of cancer. This program was developed to improve access to genetic services and identify high risk individuals in our community. The detection rate for our population in this small pilot was 5.7% which is comparable to expected. This pilot program identifies an important role for interdisciplinary approach to genetic services.
Title: Tamoxifen and contralateral breast cancer (CBC) risk for BRCA1 and BRCA2 mutation carriers: An updated analysis of data from the Kathleen Cuningham Foundation consortium for research into familial breast cancer, the International BRCA1 and BRCA2 Carrier cohort study and the breast cancer family registry

Body: Background: Findings from an analysis published in 2013, using combined retrospective and prospective data pooled from 3 cohort studies, were consistent with tamoxifen use after 1st breast cancer (BC) being associated with reduced CBC risk for both BRCA1 and BRCA2 mutation carriers, although the analysis of prospective data alone (based on 100 incident CBCs) gave inconclusive results. The association did not differ by estrogen receptor (ER) status of the 1st BC, suggesting that tamoxifen may be a useful secondary BC prevention agent for mutation carriers regardless of the ER status of their 1st BC. The aim of this updated analysis was to assess these associations after incorporating data from an additional 1,279 mutation carriers and with further follow-up providing 153 additional prospective CBC events. Methods: Eligible women were BRCA1 and BRCA2 mutation carriers diagnosed with unilateral BC since 1970 and with no other invasive cancer or tamoxifen use before their 1st BC. They were followed up from their 1st BC (or, for the prospective analysis, from the later of recruitment and 1st BC diagnosis) to the development of CBC or censoring (at contralateral mastectomy, death or loss to follow-up). Hazard ratios (HRs) for CBC associated with tamoxifen use were estimated using Cox regression, adjusting for year and age of diagnosis, country and bilateral oophorectomy; analyses were also stratified by ER status of the 1st BC. Results: This 2017 analysis includes 3,743 mutation carriers (BRCA1 2,343; BRCA2 1,400) with 21,436 person years of follow-up. Compared with the 2013 analysis, the strengths of the inverse associations were attenuated after including the additional data.
### Prospective

<table>
<thead>
<tr>
<th>Tam 1st BC</th>
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</tr>
</thead>
<tbody>
<tr>
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<td>46</td>
<td>1.00</td>
<td>191</td>
<td>21</td>
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<td>35</td>
<td>0.68 (0.40-1.15)</td>
<td>0.15</td>
<td>235</td>
<td>13</td>
</tr>
</tbody>
</table>

*Combined = retrospective and prospective, N=number, BRCA1 & BRCA2=mutation carriers, Tam 1st BC= Tamoxifen for 1st Breast Cancer

In this updated prospective analysis, the inverse association between tamoxifen use for 1\textsuperscript{st} BC and CBC risk was most apparent for women with ER positive 1\textsuperscript{st} BC, especially for BRCA2 mutation carriers: BRCA1 ER positive HR=0.45 (95% CI 0.17-1.22, p=0.12), BRCA1 ER negative HR= 0.87 (95% CI 0.45-1.67, p=0.67), BRCA2 ER positive HR=0.33 (95% CI 0.15-0.74, p<0.007), BRCA2 ER negative HR=1.12 (95% CI 0.27-4.70, p=0.88).

**Conclusions:** Tamoxifen use for 1\textsuperscript{st} BC might reduce CBC risk for mutation carriers, but predominantly for those with an ER positive 1\textsuperscript{st} BC. These data do not support use of tamoxifen to prevent CBC for mutation carriers with ER negative BC.
**Title:** Presence of atypia in ductal lavage and risk of subsequent breast cancer in a prospective study

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**Body:** Background: Atypical hyperplasia is considered a nearly obligate precursor of breast cancer and is associated with a higher risk of developing breast cancer (BC). Attempts to improve early detection of breast cancer and to provide individualized breast cancer risk assessment would greatly benefit from sampling cellular material from the target tissue. Ductal lavage (DL) is a minimally invasive technique which provides adequate material to detect atypical cells in mammary ducts. However, long term data of the association between atypia from ductal lavage and BC risk are lacking. We studied the prevalence of atypia in DL in different risk categories and its ability to predict BC development in women at risk. Methods: From March 2000 to July 2012 we performed DL in a consecutive series of 348 women with median age of 45 years (range 19-74) at increased BC risk based on the following characteristics: 5 yrs Gail model > 1.66% or > 10% probability of BRCA mutation (n = 155), history of contralateral BC (CBC, n = 161), presence of a BRCA pathogenic variant (n = 32). We analyzed the presence of atypical cells in the baseline specimen of ductal lavage and in repeated lavage and observed their evolution during follow-up. Results: The procedure was safe and well tolerated in most women, with pain and discomfort preventing the procedure in 5.4% of subjects. Overall, 126 (36%) women had atypia at baseline, with a prevalence of 32%, 39%, and 41% in the Gail, CBC and BRCA groups, respectively (p = 0.38). The overall prevalence of atypia considering all visits was 44% (range 36-51). After a median follow up of 6 years, cumulative BC events were 8% in women without atypia versus 14% in those with atypia (log-rank p = 0.08). In the highest risk groups (CBC and BRCA pathogenic variants), the number of BC events was 16 (21%) in women with atypia versus 11 (10%) in women without atypia (p = 0.02 after adjustment for age). Conclusions: Our findings suggest that cytologic atypia in the fluid obtained by DL predicts subsequent BC in women at increased risk, providing individual risk assessment. The reversal of atypia in DL should be evaluated as a surrogate biomarker of BC therapeutic prevention. Supported by: Associazione Italiana per la Ricerca sul Cancro (AIRC), Lega Italina per la Lotta contro i Tumori (LILT), AVON Foundation for Women.
**2017 San Antonio Breast Cancer Symposium**

**Publication Number:** P3-10-03

**Title:** Effects of guideline-concordant treatment on ED visits, hospitalizations, and cost in metastatic breast cancer

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**Body:**

**Purpose:** The National Comprehensive Cancer Network (NCCN) developed treatment guidelines that have directed care of patients with cancer for over 20 years. Receipt of treatment according to these guidelines is increasingly recognized as a marker of high quality care. A knowledge gap exists regarding concordance of treatment regimens for metastatic breast cancer with NCCN guidelines, as well as the potential impact of this concordance (or lack thereof) on resource utilization and costs – an issue that assumes significance in the new era of value-based healthcare.

**Methods:** From 2007-2013, women with de novo (n=988) or recurrent, treated metastatic breast cancer (n=5,651) were evaluated for concordance of first-line systemic therapy with NCCN guidelines within the SEER–Medicare linked database. Types of non-concordant treatments were reviewed and categorized. Outcomes include monthly rates of ED visits, monthly rates of hospital admissions, total overall and Medicare costs, and mortality. Specific (hospitalizations, antineoplastic agents, growth factor) and total costs to Medicare (excluding home health, hospice, skilled nursing facility) were calculated from initiation of treatment until death or available follow-up and examined by concordance status. Part D costs were excluded because costs are shared by Medicare, other payers, and patients. Cox regression was used to evaluate mortality risk. Student's t-tests, generalized linear models, and generalized mixed effects models were utilized to evaluate the relationship between concordance status and outcomes.

**Results:** We previously reported the prevalence of non-concordant first-line systemic therapy for de novo metastatic breast cancer (19%) and recurrent metastatic breast cancer (18%). The adjusted risk of mortality was comparable by concordance status. In the current analysis, non-concordant treatments were associated with a 9% increased rate of ED visits and a 7% increased rate of hospitalizations (p<.01). Total Medicare cost for patients receiving concordant and non-concordant treatments was $79,372 and $109,471, respectively (p<.001). Significant cost differences were found when comparing patients receiving concordant and non-concordant treatments by antineoplastic agents ($14,256 vs $24,817, p<.001) and growth factor ($1,754 vs $3,414, p<.001). A trend toward lower cost attributed to hospitalizations was observed for patients receiving concordant treatment compared to those receiving non-concordant treatment ($28,113 vs $34,134, p=.06). Overall, hospitalizations, antineoplastic agents, and growth factor accounted for 56% of total Medicare costs. Average monthly Medicare costs were higher for non-concordant patients by $1,761 (p<.01).

**Conclusions:** While not associated with increased overall mortality, non-concordant treatment is associated with higher health care utilization rates and cost. Increased costs attributed to non-concordant care were largely driven by antineoplastic agents and growth factor use. These findings may have policy implications for payment reform initiative, in particular pathway programs which aim to reduce variability in care and spending on medications.
Title: Obesity and adipose inflammation in men with breast cancer

Samantha Williams¹, Julia C Parrish², Xi K Zhou³, Hanhan Wang³, Anneloor Dierickx⁴, Ayca Gucalp¹, Andrew J Dannenberg³ and Neil M Iyengar¹. ¹Memorial Sloan Kettering Cancer Center, New York, NY; ²University of Washington School of Medicine, Anchorage, AK; ³Weill Cornell Medicine, New York, NY and ⁴University of Ghent, Ghent, Belgium.

Body: Background: Elevated body mass index (BMI) is associated with increased risk of hormone receptor (HR)-positive breast cancer in postmenopausal women and worsened outcomes after breast cancer diagnosis. These observations may be partly attributable to adipose inflammation, which is prevalent in the breasts of obese women and is associated with worsened breast cancer survival. In men, some studies have reported obesity to be a risk factor for breast cancer, however the biologic links are not well characterized. Whether adipose inflammation occurs in male breast tissue has not been previously reported. Here we examined the relationships among pre-diagnosis BMI, adipose inflammation, and breast cancer features in men.

Methods: Males diagnosed with stage 0 – III breast cancer who underwent mastectomy at Memorial Sloan Kettering (MSK) between August 1991 – November 2011 were included in this retrospective cohort study. Pre-operative BMI was categorized as normal or underweight (<25), overweight (25 – 29.9), obese (≥30), or morbidly obese (≥40 or ≥35 + co-morbidity). Archived breast tissue was subjected to CD68 immunohistochemistry to detect adipose inflammation, defined by the presence of dead or dying adipocytes surrounded by macrophages – known as crown-like structures of the breast (CLS-B). Clinicopathologic associations with BMI and CLS-B were analyzed by logistic regression and Fisher's exact test.

Results: A total of 141 men were included; median age 63 (range 23 – 96). By BMI category, 25 were normal or underweight, 65 overweight, and 51 obese – of which 19 were morbidly obese. Only 11 men had known BRCA1/2 mutations. Median age at diagnosis was 69 in normal/underweight men versus 63 in obese men and 51 in morbidly obese men (P≤0.05). Among those with invasive tumors, average tumor size was 1.50 cm (± 0.84) in normal/underweight men versus 2.04 (±0.81) in morbidly obese men (P≤0.05). Archived breast tissue was available from 92 (65%) men. Breast adipose inflammation was present in 55 (60%) men, and average BMI was 31 (±8) versus 28 (±5) in men with versus without inflammation, respectively (P=0.07).

Conclusions: Obesity is associated with early onset breast cancer in men. Morbidly obese men were diagnosed with breast cancer at an even younger age and had larger tumors than normal weight individuals. These findings support further studies to investigate mechanisms, such as adipose inflammation, through which obesity may promote breast cancer in men.
Title: Normal breast biopsies reveal an "active" transcriptome associating with higher breast cancer risk (Gail) scores and increased IGF-1 growth factor expression

Christopher Benz¹, Christina Yau¹, Stephen Benz², Gregor Krings³ and Mark Powell⁴. ¹Buck Institute for Research on Aging, Novato, CA; ²NantOmics LLC, Culver City, CA; ³University of California, San Francisco, San Francisco, CA and ⁴Zero Breast Cancer, San Rafael, CA.

Background: Recent studies have identified at least two different transcriptional subtypes of benign human breast tissue (Haakensen et al., 2011), including an “active” subtype associated with increased risk of later life mortality from breast cancer (Troester et al., 2016). However, previous studies used "normal" breast samples from cosmetic surgery, biopsies from abnormal mammograms, or cancer-adjacent tissue. The present study evaluates normal breast transcriptome phenotypes from healthy women who donated for this study purpose only.

Methods: 200 formalin-fixed paraffin-embedded (FFPE) breast tissue biopsy samples were analyzed from healthy, parous non-Hispanic white women ranging in age from 27–66 (median = 45) with no prior history of breast cancer (<16% had prior breast biopsies), who donated to the Komen Tissue Bank (KTB) and provided questionnaire data enabling calculation of breast cancer risk (Gail) scores. Extractable RNA from FFPE sections sufficient for RNA sequencing (~100ng) was obtained from 145/200 donor samples; digitized H&E analysis confirmed normal breast histology. Transcript levels were quantified using RSEM and normalized; 2558 genes were used for unsupervised hierarchical clustering to identify “active” vs. “inactive” normal transcriptome subtypes as previously described (Roman-Perez et al., 2012), and these subtypes were compared for risk scores and IGF1R and IGF-1 gene expression levels and their respective signatures (Creighton et al., 2008; Mu et al., 2012).

Results: As expected, the normal histologic composition of these KTB samples varied considerably with mean % adipose area twice that of fibrous area, and epithelial content averaging <5%. Unsupervised gene expression analysis produced two primary clusters, with 31% of donor samples showing the “active” transcriptome phenotype. These “active” breast samples came from women with significantly higher Gail scores (Wilcoxon rank sum p=0.007) and showed significantly different expression levels in 12 of 17 IGF-1/IGF1R pathway genes (FDR-corrected p values) including higher IGF-1, IGF-2, IRS1, IRS2, IGF2R, IGFBP3, IGFBP5, IGFBP6, and IGF2BP3 levels, and lower IGF1R, IGFBP2, and IGFBP3 transcript levels. A multigene signature associated with IGF-1 induction was consistent with the 2-fold significant increase in mean IGF-1 mRNA levels seen in this “active” subset; likewise, an independent multigene signature associated with IGF1R activation was consistent with the 0.84-fold significant reduction in mean IGF1R mRNA levels observed in this same subset.

Conclusion: Over 30% of healthy adult women with histologically normal breast tissue carry an "active" breast transcriptome phenotype previously linked to co-existent breast cancer and now shown to be associated with increased future risk of developing breast cancer assessed by Gail score. This “active” transcriptome phenotype is characterized by increased endogenous IGF-1 activity, a known breast cancer promoting growth factor, along with an inverse reduction in IGF1R expression and activity as previously seen in some breast cancers. Further morphologic and molecular characterization of this risk-associated subset of normal breast tissues is underway.
Title: What is the latest evidence on diet, nutrition, physical activity and cancer – key findings from the WCRF/AICR continuous update project

Inger Thune¹,², Kate Allen³, Rachel L Thompson³, Martin J Wiseman³, Panagiota Mitrou³ and Deirdre McGinley-Gieser⁴. ¹Oslo University Hospital, Oslo, Norway; ²University of Tromsø, Tromsø, Norway; ³World Cancer Research Fund International, London, United Kingdom and ⁴American Institute for Cancer Research, Arlington, VA.

Body: Introduction:
Since 1997, World Cancer Research Fund (WCRF) International and the American Institute for Cancer Research (AICR) have been at the forefront of synthesizing and interpreting the accumulated scientific literature on the link between diet, nutrition, physical activity and cancer, and deriving evidence-based Cancer Prevention Recommendations. The 2007 WCRF/AICR 2nd Expert Report, using a robust method and systematic literature reviews, was a landmark in the analysis of evidence linking diet, body weight and physical activity to cancer and led to the establishment of the Continuous Update Project (CUP). New findings from the CUP systematic review have been released for 12 cancers, and updated findings for breast cancer have just been released. The 2018 WCRF/AICR 3rd Expert Report, to be published in 2018, will include a new review of the WCRF/AICR Cancer Prevention Recommendations.

Methods:
The review was conducted as part of the Continuous Update Project (CUP).
The research team at Imperial College London searched PubMed for relevant prospective studies up to April 30, 2015. Dose-response meta-analyses were conducted and summary relative risks (RR) were calculated using a random effects model. Analyses comparing the highest versus the lower categories were also conducted. An international panel of experts (CUP Panel) reviewed the evidence and drew conclusions.

Results:
The updated Breast Cancer Report included 119 studies from around the world, comprising more than 12 million women and over 260,000 cases of breast cancer.
The latest evidence suggests that vigorous physical exercise reduces your risk of breast cancer before menopause by 17%. In addition, the report reconfirms the link between physical activity and decreased risk of breast cancer after menopause. Taking at least 30 minutes of moderate physical activity, such as brisk walking, or 15 minutes of vigorous physical activity each day can reduce your risk of breast cancer after menopause by 10%. Greater body fatness and weight gain as an adult does increase the risk of breast cancer after menopause. However, in contrast we observed greater body fatness in young adulthood may reduce breast cancer risk both before and after menopause.
The report found strong evidence that drinking just the equivalent of a small glass of wine or half a pint of beer a day (about 10g alcohol content), could increase your pre-menopausal breast cancer risk by 5% and your post-menopausal breast cancer risk by 9%.

Conclusions:
Recent evidence has resulted in new findings and changes to the CUP Panel's conclusions from the 2010 Breast Cancer Report with regard to physical activity and body fatness at different life periods. In addition, new evidence confirmed their judgements on alcohol.
The CUP provides a unique resource synthesizing epidemiological and other evidence on diet, nutrition, physical activity and cancer, to facilitate related research, and underpin advice to the public and policy-makers.
Evidence is accumulating that the degree of adherence to WCRF/AICR recommendations is associated with lower mortality of cancer overall, of specific cancers and of all-cause mortality.
Title: Uptake of risk reducing measures in healthy BRCA 1-2 mutation carriers from Northern Italy: Outcomes of 9 years of close-surveillance. Experience of the hereditary cancer clinic in the region of Veneto, Italy

Antonella Rastelli1,2, Fabio Azzolin1, Silvia Tognazzo1, Elisa Alducci1 and Stefania Zovato1. 1IOV (Istituto Oncologico Veneto), Padova, Italy and 2Washington University School of Medicine, Saint Louis, MO.

Body: Background: There are several options for cancer reduction in women with a BRCA 1-2 mutation, including prophylactic surgery, chemo-prevention and close surveillance. Each of these measures offers varying levels of effectiveness with prophylactic mastectomy (PM) and oophorectomy (PO) providing the greatest risk reduction for the development of breast and ovarian cancer. The uptake of preventive procedures, however, differs according to country, not only in relation to access to care, but also cultural preferences. Here we present data on the experience of the Hereditary Cancer Center at the Istituto Oncologico Veneto, which is one of the few centers in Northern Italy to offer not only genetic testing, but also long-term follow-up to healthy carriers of the BRCA 1-2 genes. Methods: 106 healthy women were identified within a cohort of BRCA positive individuals followed since 2008. Upon learning of their BRCA mutation carrier status, they all agreed to enroll in a long-term protocol of close-surveillance. All women were also informed about risk-reducing surgery such as PO and PM. Results: The age in this sample ranged between 20 and 77 years with a median of 41.5. The average follow-up time was 4.3 +/-2.4 years. We observed that PO was more commonly accepted (41% 44/106) than PM (11% 12/106). The mean age at the time of PO was 47.3. In the PM group, half of the women decided to undergo such procedure only after being diagnosed with breast cancer. The mean age at the time of PM in the healthy subjects was 41.8 years. Over the follow-up period, 9.4% of the patients (10/106) developed BRCA related malignancies, of which 9 breast and 1 ovarian cancer. Of the 9 breast events, 2 were diagnosed at stage 0, 2 at stage I, 3 at stage II, and 2 at stage III. All cancer cells were aggressive (grade 3). Of the 2 stage III breast tumors, 1 was diagnosed on the very 1st breast MRI screening, and the other after an interval of 3 years due to the patient being pregnant and then breastfeeding. The 3 cases of stage II breast cancer, despite being 1 cm or less in size, already had node involvement. Both stage III breast tumors were triple negative in BRCA 1 carriers. The other breast cancers were ER+ and none was Her2+. Of the ER+ breast events 4 occurred in BRCA2 and 2 in BRCA1 carriers. Except for the 2 Stage 0 tumors, which arose in women who had a previous oophorectomy, all the other breast malignancies developed in carriers who still had their ovaries and were premenopausal. The one serous ovarian cancer was diagnosed at Stage II in a 77 years-old BRCA1+ woman. Conclusions: In this sample of healthy northern Italian women carriers of BRCA mutations, close to 10% developed a tumor within 5 years of follow-up with an aggressive cell phenotype. The more accepted method of risk-reduction in this group of women was PO as compared to PM. A protocol of close-surveillance allows for an early stage diagnosis in about half of the women who develop a breast tumor.
Title: Breast cancer in octogenarians: Presentation and treatment affect mortality

Jason P Wilson¹, Vijayakrishna Gadiyaram¹ and Peter W Blumencranz¹. ¹Morton Plant Mease Hospitals, Clearwater, FL.

Body: Background: Breast cancer incidence increases in age. Unfortunately, elderly patients are often under-treated and under-diagnosed affecting their overall survival with limited availability of clinical trial data.

Patients and Methods: We used data from our cancer registry and our state cancer registry to evaluate cases of breast cancer diagnosed between the years 2005 and 2010 with follow up data available to date to examine the method of diagnosis (mammogram vs. palpable abnormality), age at diagnosis, stage at diagnosis, type of surgery, receptor status, treatment received, and survival. We then were able to use our state database which contained data regarding causality of death. Based on the data available we were able to evaluate the impact of the method of diagnosis and treatment on patient mortality.

Results: A total of 495 octogenarian breast cancer patients were diagnosed between 2005 and 2010. The median age at diagnosis was 85 with a range of 81-102. Out of the 495 patients 55% were diagnosed with a mammogram and 41% by palpable abnormality. The patients who presented with a palpable mass were more likely to die of breast cancer (26%) than the patients who presented with a screening abnormality (9%). The patients with a screening abnormality also had a longer survival (30.0 months) among patients who died from breast cancer as opposed to those who presented with a palpable abnormality who died from breast cancer (16.7 months). While the number of patients dying from other causes was similar (65% screened vs. palpable) the patients with palpable findings had a shorter survival (30.5 months) compared to those detected by screening (48.9 months).

Table 1: Comparison of outcomes between screen detected and physical exam detected breast cancer patients

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Screened</th>
<th>Palpable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Died of Breast Cancer</td>
<td>9% (30.0 months)</td>
<td>26% (16.7 months)</td>
</tr>
<tr>
<td>Died of Other Causes</td>
<td>65% (48.9 months)</td>
<td>60% (30.5 months)</td>
</tr>
<tr>
<td>Still Alive</td>
<td>25.6% (66.9 months)</td>
<td>10% (51.0 months)</td>
</tr>
</tbody>
</table>

There was an improvement in survival among patients who presented with a palpable mass and were treated with endocrine therapy (17.3 vs 11%). This improvement did not extend to the screened population (24.3% vs 29%), although the patients who died from breast cancer had an overall longer survival (35.4 months vs 27.3 months). There was also an improvement in survival among patients who received radiation therapy in both the screened (33% vs. 22%) and palpable (22.5% vs 11.1%) groups. Only 6% of patients in the entire study received chemotherapy.

Effect of presentation and treatment on survival

<table>
<thead>
<tr>
<th>Modality</th>
<th>Screened/Treated</th>
<th>Palpable/Treated</th>
<th>Screened/ No Treatment</th>
<th>Palpable/No Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiation</td>
<td>33% (29.6 months)</td>
<td>22.5% (10 months)</td>
<td>22% (27 months)</td>
<td>11.1% (20 months)</td>
</tr>
<tr>
<td>Endocrine</td>
<td>24.3% (35.4 months)</td>
<td>17.3% (15.7 months)</td>
<td>29% (27.3 months)</td>
<td>11% (18.5 months)</td>
</tr>
</tbody>
</table>

Conclusion: Our data support continued use of screening mammography in patients who are at good surgical risk into their 80's. The patients who were diagnosed with symptoms fared worse than the patients who had their cancer discovered on routine
imaging. There was an improvement in patients who were treated with adjuvant endocrine and radiation therapy although the rationale behind providing or excluding treatment cannot be discerned from this retrospective registry study.
Title: The association age-related parity and HER2 over-expression in breast cancer

Soo Youn Bae¹, Jiyoung Kim², Ji Young You¹, Seung Pil Jung¹ and Jeong Won Bae¹. ¹Korea University Anam Hospital, Seoul, Korea and ²Jeju National University of Hospital, JeJu, Korea.

Body: Backgrounds

Reproductive factors, such as age at menarche, parity, age at first birth, and breast feeding, are known to be associated with risk of breast cancer. Recent studies show the association between the reproductive factors and the molecular subtypes of breast cancer, especially in luminal-like subtypes. Nulliparity is known a risk factor of breast cancer, multiparity is considered a protective factor. However, pregnancy associated breast cancers show the high expression of HER2, Therefore, we hypothesized the number of birth (multiparity) is correlated with the over-expression of HER2.

Methods

We reviewed the database of 2,594 breast cancer patients were over 20 years of age, diagnosed between January 2000 and April 2017 at Korea University of Anam Hospital, Seoul, Korea. According the number of birth, nulliparous (the number of birth = 0; 271 patients), primiparous (the number of birth = 1; 420 patients) and multiparous (the number of birth ≥2; 1,903 patients) groups were classified.

Results

The median age (range) of nulliparous, primiparous and multiparous groups was 43 (22-77), 48 (27-87) and 51 (26-86) years (P<0.001). In univariate analysis, the number of birth was associated with the expression of ER and HER2, nulliparous patients showed higher ER positivity (P=0.013) and multiparous patients showed higher HER2 overexpression (P=0.048). There was no association with PR and Ki-67 level. In logistic regression with age, the parity was negative correlation with ER positivity (OR 0.83, 95% CI 0.72-0.95, P=0.010) and positive correlation with HER2 overexpression (OR 1.18, 95% CI 1.02-1.36, P=0.021) and multiparity was higher HER2 overexpression (OR 1.72, 95% CI 1.12-2.63, P=0.013), there was no difference in HER2 overexpression between nulliparous and primiparous patients.

Conclusion

Parity was associated with ER and HER2 expression, multiparous patients were associated with ER negativity and HER2 overexpression, especially in premenopausal women. This suggests that pregnancy could affect risk of breast cancer, especially in HER2 positive breast cancer subtype (ER- HER2+ tumors), before menopause. We need further investigation and evaluation.
Title: Triple negative breast cancer in young Peruvian patients: 15-years' experience in a public hospital

Zaida Morante¹, Gabriel De la Cruz-Ku³, Joseph Pinto², Jhajaira Araujo¹, Eduardo Eyzaguirre³, Antonella Saavedra³, Maria Lujan³, Daniel Enriquez¹, Magno Ramirez³, Hugo Fuentes¹, Silvia Neciosup¹ and Henry Gomez¹. ¹Instituto Nacional de Enfermedades Neoplasicas, Lima, Peru; ²Oncosalud, Lima, Peru and ³Universidad Cientifica del Sur, Lima, Peru.

Body: Background: The incidence of breast cancer (BC) is low in young women and is characterized by a high prevalence of triple-negative tumors, representing a group of high risk. In this work, we describe the clinicopathological and epidemiological features of triple-negative breast cancer (TNBC) in patients aged ≤35 years.

Methods: We reviewed information of TNBC patients diagnosed at ≤35 years old and treated at the Instituto Nacional de Enfermedades Neoplasicas (between 2000 and 2014). The Cox proportional hazard model was used to identify prognostics factors for DFS and OS.

Results: In total, of 243 out 2007 cases (12.11%) were very young TNBC patients. The median follow-up was 9 years. The median age was 32 years (range: 19-35); 14.8% had obesity. A total of 40 (16.5%) patients had a family history of breast-ovary cancer (FHBOC). Regarding to the clinical-pathological characteristics, 59.4% presented T-Stages 3/4; 65.2% had nodal involvement and 7 patients (2.9%) had bilateral BC. Most of patients were diagnosed at Clinical Stage (CS) III (50.8%). The most common histological subtype the was ductal invasive carcinoma (92.1%), followed by medullar (4.5%) and lobulllar (1.7%). A high histological grade was frequent (84.7%), while 71 cases (49%) were diagnosed with vascular permeation and 55 (48.2%) with macrometastasis in lymph nodes. The majority of patients underwent mastectomy (60.2%) compared to lumpectomy (39.8%). Locoregional relapse and distant metastasis were reported in 30.9% and 49.4% of cases, respectively. The most frequent sites of metastases were lung (14.8%) and brain (11.5%). In the multivariate analysis, only N3 stage was associated with a poor outcome in terms of (N0 vs N3, HR=7.89, 95%CI:2.76-22.56, p<0.001). Variables associated with the risk of death were N stage (P<0.001 for N0 vs N3), neoadjuvant chemotherapy (P<0.027), adjuvant chemotherapy (P<0.001), and radiotherapy (P=0.008).

Conclusions: TNBC in very young Peruvian women was characterized by advanced stage at diagnosis. In these patients, nodal involvement was the most important prognostic factor for DFS. It presents distinctive characteristics and poorer outcomes in terms of DFS and OS.

Table 1. Multivariate Cox Regression Analysis.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>PFS</th>
<th>OS</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95% CI</td>
</tr>
<tr>
<td>N0 vs N1</td>
<td>1.82</td>
<td>0.74-4.48</td>
</tr>
<tr>
<td>N0 vs N2</td>
<td>2.26</td>
<td>0.69-7.36</td>
</tr>
<tr>
<td>N0 vs N3</td>
<td>7.89</td>
<td>2.76-22.56</td>
</tr>
<tr>
<td>Adjuvant chemotherapy</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Neo-Adjuvant Chemotherapy</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

ns = not significant
Title: Breast cancer risk assessment and evaluation of risk-based screening practices by primary care providers: A single institution experience

Henry P Igid1, J Drew Payne1, Anita Sultan1, Sean Y Kow1, Keeley L Hobart1, Teri Nichole Payne1 and Catherine Jones1. 'Texas Tech University Health Sciences Center, Lubbock, TX.

Body: Background: Risk reduction intervention for primary prevention of breast cancer (BC) with tamoxifen, raloxifene, exemestane, and anastrazole has been well studied in high risk women. Despite this established risk reduction strategy, chemoprevention (CP) remains underutilized. In this retrospective chart review, we describe the utilization of BC risk assessment, prevention awareness, and adherence to BC screening recommendations among primary care providers in Internal Medicine (IM), Family Medicine (FM), and Obstetrics and Gynecology (OB) in our institution.

Methods: A total of 1220 electronic charts of women aged ≥35 and ≤78 who came for preventive visits in 2014 (n=1076, OB; n=59, IM; n=80, FM) were reviewed. Charts were evaluated for pertinent histories related to BC risk assessment. Gail scores were calculated for all patients based on available histories with conservative estimates in those with incomplete data recorded. Screening compliance with annual or biennial mammography was recorded.

Results: The percentage of patients having adequate histories in the chart to allow calculation of Gail score was 56.9% in OB, 9.4% in FM, and 1.7% in IM, χ²(2)=131.08, p<0.001. Age at menarche was not documented in 29.5% of OB patients, 56.5% of FM patients, and 94.5% of IM patients, χ²(2)=123.78, p<0.001. Age at first live birth or parity was not documented in 17.8%, 85.9%, and 97.7% of OB, FM, and IM patients respectively, χ²(2)=286.89, p<0.001. Calculation of the Gail score yielded a total of 149 patients (12.2%) who are considered high-risk and potentially eligible for CP. No patients were offered CP as part of their care. IM physicians had significantly higher adherence to BC screening recommendations by NCCN, ACS, and USPSTF (55.36%-81%) compared with OB (45.74%-68.3%), and FM physicians (45.88%-68.6%), (p<0.001). No patients had documented risk-based BC screening even among those found to be high-risk.

Differences between low vs high-risk patients by Gail score

<table>
<thead>
<tr>
<th></th>
<th>High Risk (Gail ≥ 1.67)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (n=1220)</td>
<td></td>
</tr>
<tr>
<td>Gail score, M (SD)</td>
<td>1.101 (0.68)</td>
<td></td>
</tr>
<tr>
<td>Age, M (SD)</td>
<td>48.51 (9.17)</td>
<td></td>
</tr>
<tr>
<td>Age at 1st mammogram, M (SD)</td>
<td>45.13 (7.77)</td>
<td></td>
</tr>
<tr>
<td>Age at menarche, M (SD)</td>
<td>12.62 (1.49)</td>
<td></td>
</tr>
<tr>
<td>Age at first live birth, M (SD)</td>
<td>24.34 (5.85)</td>
<td></td>
</tr>
<tr>
<td>Number of first degree relatives with history of BC, n (%)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1036 (84.9)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>143 (11.7)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>9 (0.7)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>32 (2.6)</td>
<td></td>
</tr>
<tr>
<td>History of breast biopsy, n (%)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1150 (94.3)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>64 (5.3)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>6 (0.5)</td>
<td></td>
</tr>
<tr>
<td>Number of biopsies</td>
<td>0.021</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>57 (89.1)</td>
<td></td>
</tr>
</tbody>
</table>
P-value compares two groups based on Gail score cutoff (≥1.67).

**Conclusions**: BC risk assessment, risk-based screening, and use of BC chemoprevention is not adequately practiced by primary care providers. Primary care providers' adherence to BC screening recommendations was closest to USPSTF guidelines (68.3% to 81.1%) and did not follow risk-based screening recommendations. Rates of adherence to chemoprevention and mammogram screening recommendations approximated national data.
2017 San Antonio Breast Cancer Symposium

Publication Number: P3-10-13

Title: Bilateral breast cancer in China: A 10-year single-center retrospective study

Huiping Li1, Hanfang Jiang1, Ruyan Zhang1, Bin Shao1, Ying Yan1, Ran Ran1 and Lijun Di1. 1Peking University Cancer Hospital & Institute, Beijing, China.

Body: BACKGROUND: Women with unilateral breast cancer are at increased risk for developing contralateral disease. The objective of the single-center retrospective study was to evaluate the incidence of bilateral breast cancer (BBC) and to analyze the clinicopathological characteristics for BBC in China.

METHODS: We retrospectively reviewed the electronic medical records of 3924 female patients with breast cancer consecutively treated at the department of Breast Oncology in the Peking University Cancer Hospital between 2006 and 2016. BBC was categorized as synchronous (within 6 months) or metachronous bilateral breast cancer (after 6 months of a first tumor). Patients with BBC were identified according to the criteria described by Chaudary et al. Patients with stage IV first breast cancer, and those who were found to have distant metastases between the first and second primary breast cancer were excluded. Analyses of demographic, clinicopathologic, and treatment characteristics were done between sBBC and mBBC.

RESULTS: The incidence of BBC in our population was 3.2% (127 of 3924). Of those, 2.5% (99 of 3924) were metachronous bilateral breast cancer (mBBC), and 0.71% (28 of 3924) were synchronous bilateral breast cancer (sBBC). The overall median age of the patients at first diagnosis was 45 years (range, 27-81 years). The age of onset of the first mBBC was significantly younger than that of sBBC (p = 0.027). In mBBC subgroups, the median time interval between first and second tumors was 68 months (range, 7-342 months), and 80.8% of the second tumor were diagnosed within 10 years of the diagnosis of the first tumor. A positive family history of breast cancer was found in 25% of sBBC and 9.1% of mBBC (p = 0.025). In ER-negative first tumor of mBBC, 56.1% of the second tumor were ER-positive. Mastectomy was the commonest surgery performed in these patients.

CONCLUSIONS: Our results confirmed the necessity of screening contralateral breast at the diagnosis of unilateral breast cancer and following-up for Chinese patients with breast cancer.
Body: Background: Despite available treatment for early-stage breast cancer (BC), 15%-25% of patients with early-stage human epidermal growth factor receptor 2–positive (HER2+) BC eventually experience recurrence after initial treatment. The prognosis for women with HER2+ disease recurrence is poor. Most recurrences involve incurable metastatic disease. In the US, the total cost to society attributable to metastatic BC of any subtype was $12.2 billion accrued over 5 years, or $2.4 billion per year ($98,571 per patient-year). Treatment-related cost, 57% of total costs, was the largest contribution, with over $1.0 billion per year. The purpose of this study was to assess the clinical and economic burden of recurrence in patients with early-stage HER2+ BC.

Methods: We conducted two systematic literature reviews (SLRs) and one targeted literature review (TLR) in PubMed, Embase, and Cochrane databases. The SLRs (no publication date limit; clinical SLR conducted on November 8, 2016; economic SLR conducted on October 25, 2016) searched for randomized clinical trials of neratinib and other treatments and economic data (models, utility, resource use, and cost), and the TLR (publications published from January 2006 to September 2016) searched for burden-of-illness studies in early-stage HER2+ BC.

Results: A total of 4,708 abstracts (2,649 clinical SLR; 969 economic SLR; 1,090 TLR) were identified from all searches, and full-text review was conducted for 796 articles (507 clinical SLR; 151 economic SLR; 138 TLR). Of these, 159 (72 clinical SLR; 33 economic SLR; 54 TLR) followed protocol-specified criteria for inclusion. Based on clinical trials in the neoadjuvant and/or adjuvant setting, disease-free survival rates at 4 years ranged from 78% to 90%. HER2-targeting adjuvant regimens such as lapatinib added to trastuzumab and extending trastuzumab to 2 years have been unsuccessful in reducing the risk of recurrence. Women who had a recurrence, regardless of HER2 status, reported significantly poorer functioning on various quality of life (QoL) domains compared with women who remained disease free. All patients with early-stage BC, regardless of HER2 status, diagnosed with their first recurrence experienced cancer-related distress and no improvement in QoL (physical health and functioning) after 1 year. In the US, the total expected per-patient costs for all BC, regardless of HER2 status, over 10 years was $53,454 with metastatic recurrence, $61,601 with locoregional recurrence, and $61,188 with contralateral recurrence as compared with $42,005 (background costs) with no recurrence (2004 US $). The overall cost of recurrence in women with HER2+ BC in the US was estimated to be $240 million to $1.7 billion over the lifetimes of each 1-year cohort of 7,298 patients (2008 US $).

Conclusions: These results identified few studies on patients with early-stage HER2+ BC and suggest that future studies are warranted. Recurrence in women with HER2+ BC is associated with decreased QoL and high costs. After adjuvant therapy, there is still risk of recurrence, thus the clinical and economic burden remains. There is an unmet medical need in early-stage HER2+ BC, and new therapies are needed to reduce the risk of recurrence.
**2017 San Antonio Breast Cancer Symposium**

**Publication Number:** P3-10-15

**Title:** Stellate cells and mesenchymal stem cells in benign mammary stroma are associated with risk factors for breast cancer

Håkan L Olsson¹², Bjorn L Isfoss¹, Bo Holmqvist¹ and Helena Jernstrom². ¹Clinical Sciences, Lund University, Lund, Sweden and ²Clinical Sciences, Lund University, Lund, Sweden.

**Body:**

**Background:** It is not known whether stromal cells in benign breast tissue can mediate risk of breast cancer. We recently described aldehyde dehydrogenase 1 A1 (ALDH1) positive (+) cells in morphologically normal breast stroma of premenopausal women, and the data indicated that their distribution is associated with clinical risk factors for breast cancer. The aim of the present study was to define the identities of these cells using histological and immunohistological methods, and to investigate associations between those cells and hormonal and genetic risk factors in pre- and postmenopausal women.

**Methods:** Stroma of morphologically normal tissue was analyzed in samples from 101 well-characterized women whose breasts had been operated. Morphology and immunolabeling were applied to determine cell identities based on the putative stem cell markers ALDH1 and stage-specific embryonic antigen-3 (SSEA3), and immunophenotypes indicating mast cells or stellate cells. The results were compared with the patients' risk factors using regression analysis (two-tailed).

**Results:** ALDH1+ round/oval cells were associated with low parity in BRCA1/2 carriers ($p = 0.022$), while in non-BRCA1/2-carriers they were negatively associated with nulliparity ($p = 0.057$). In premenopausal women ALDH1+ round/oval cells were associated with family history ($p = 0.058$). SSEA3+ round/oval cells were morphologically and immunohistologically consistent with multilineage stress-enduring (Muse) cells, and these cells were independently associated with the breast cancer risk factors low parity ($p = 0.015$), family history ($p = 0.021$), and hormone use after menopause ($p = 0.032$). ALDH1+ spindle-shaped/polygonal cells were immunohistologically consistent with stellate cells, and were negatively associated with family history of breast cancer ($p = 0.001$).

**Conclusion:** This study identified novel stromal cell types in benign breast tissue that have a potential for stratifying women for breast cancer risk.
2017 San Antonio Breast Cancer Symposium

Publication Number: P3-10-16

Title: Competing mortality risks: Cardiovascular risk versus risk of metastatic disease in breast cancer patients receiving adjuvant therapy

Lisa Prior1, Hannah Featherstone1, Killian Nugent1, Marvin Lim1 and Catherine Kelly1. 1Mater Misericordiae University Hospital, Dublin, Ireland.

Body: Background
Breast cancer is the most commonly diagnosed cancer in women in Ireland. The 5 year net survival is over 80%. Most women diagnosed with breast cancer do not die from the disease and instead cardiovascular disease (CVD) remains the most frequent cause of death. Many breast cancer patients are older and have established CVD risk factors. They are at further risk of a cardiovascular (CV) event due to exposure to anthracyclines, HER2 targeted agents, endocrine therapy and radiotherapy. In this study, we aimed to compare the 10 year risk of breast cancer mortality versus that of a CV event or death in an oncology day ward population at our centre through the use of online prognostic risk calculators. Furthermore, we sought to investigate the predicted outcome of lifestyle changes on their overall CV risk and level of interest in addressing these risk factors.

Methods
This was a cross cohort study of all breast cancer patients attending for adjuvant chemotherapy at our institution from September 2015 to September 2016. To calculate patients’ 10 year CVD risk, we used the JBS3 online calculator. Using this calculator we could also predict patient’s lifetime CV risk. To calculate patients’ 10 year mortality risk from breast cancer we used NHS predict! These calculators incorporate several variables which we identified from chart review, the use of questionnaires and the performance of a serum lipid test.

Results
101 subjects were identified. All patients (pts) completed questionnaires. Eighteen pts were excluded from CV risk analysis due to lack of lipid profile. Of 83 pts, 83% (n=69) were >50 years of age and 17% (n=14) pts were <50 years of age. The median age in the >50 age group (grp) was 59 and 42 in the <50 age grp. The median BMI in the >50 and <50 grp was 29.2 and 26.4 respectively. Forty-two percent (n=29) of pts in the >50 grp were smokers/ex smokers and 49% (n=7) were in the <50 grp. Twenty-nine percent (n=20) in the >50 grp were on statins and 25% (n=17) were on antihypertensives. Fifty-two percent (n=36) of pts in the >50 grp and 66% (n=10) received anthracyclines. Fifty percent in both grps received left sided radiotherapy. Ninety five percent (n=63) were on endocrine therapy in the >50 grp and 93% (n=13) in the <50 grp. Forty-one percent in the >50 grp (n=28) and 29% (n=4) of patients in the <50 grp received Trastuzumab. In the >50 grp, 13% (n=9) were found to have a high 10 year cardiovascular risk (>20% risk of CV event) and 28% were found to be at moderate risk (n=19). Ten percent of patients were found to have a CV risk that exceeded their breast cancer mortality risk (n=7). Even though the 10 year CV risk was low in patients <50, their median lifetime risk was calculated at 52%. Eighty seven percent of pts were interested in receiving advice on reducing their cardiovascular risk. The predicted median 10 year cardiovascular risk of age>50 patients in the high risk group post CV risk modification was 8.8% (+/-5.6) versus 28% (+/-8) at baseline.

Conclusion
This patient population has significant CV risk factors at baseline. Benefits predicted with CV risk intervention model and significant patient interest indicates that formalised CV risk prevention strategy is needed.
Body: Background/Objective: Biomarkers such as estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor 2 (HER2neu) are associated with the survival and recurrence of invasive breast cancer (IBC). Presence of these markers often helps to dictate treatment plans. The purpose of this study is to identify the predictors of overall survival (OS) and disease-free survival (DFS) for women diagnosed with IBC. In this study, we compared OS and DFS in the following five categories based on biomarker expression: triple-negative (TN), triple-positive (TP), HER2neu-positive, Luminal A (LA) and Luminal B (LB).

Method: We retrospectively examined 6,231 records of women diagnosed with IBC from 2006 through 2015 within the Aurora Health Care system. The majority of the women were white (90.8%) and non-Hispanic (97.4%). The women in this study were categorized in the age groups: 20-39 (4.9%), 40-59 (42.8%), 50-69 (40.7%) and 70+ years (11.6%). We used descriptive statistics for all category and numeric variables. Kaplan-Meier curve was used to compare OS and DFS in the five categories. Cox proportional hazards regression was used to identify the significant predictors of OS and DFS. We used alpha of 0.05 for all statistical tests and all statistical analysis was done using SAS 9.4.

Results: OS was 86.39% and the DFS rate was 84.43%. In multivariate analysis significant predictors of OS included age (HR=1.94 for 60-69 vs 20-39 p=0.001 and HR=5.3 for 70+ vs 20-39 p<0.001), race (HR=0.57, p=0.043 for white vs other), ethnicity (HR=0.34, p=0.032), HER2neu expression (HR=0.58, p <0.001), size of the tumor (HR=1.001 p=0.011), grade differentiation (HR=0.62 p < 0.001) and cancer stages (HR of 1.17, 4.8, 15.2 for II, III and IV vs I, p<0.001). Furthermore treatments such as surgery (HR=0.64, p< 0.001), chemotherapy (HR=0.63, p <0.001) and hormonal therapy (HR=0.48, p <0.001) showed significantly improved OS in the patients. Similarly significant predictors of DFS included, age (HR=1.8, p <0.001 for 70+ vs 20-39); PR (HR=0.70, p=0.003), HER2neu (HR=0.60, p <0.001) tumor size (HR=1.001, p=0.002), cancer stage (HR=2.7, 9.2, 25.5 for II, III, IV vs I, p<0.001); differentiation of the tumor (HR=0.75, p=0.002, moderate differentiation; well differentiated, HR=0.50, p <0.001). Moreover, the treatments such as surgery (HR=0.42, p <001) radiation, chemotherapy and hormonal therapy (HR=0.58, 0.62, 0.51 p <0.001) significantly increased DFS. Furthermore OS was better for women diagnosed with TP, LA, HER2, LB types compared to women with TN (p<0.001). Similarly the probability for DFS was higher for TP also was higher compared to the probabilities all the other categories (LA, LB, HER2 and TN p <0.05). LA had better DFS then LB, HER2 and TN. LB and HER2 had better DFS than TN (p<0.05).

Conclusion: This large study suggests that treatment based on biomarker expression (ER, PR and HER2/Neu) has improved OS and DFS for women with IBC. Increase in tumor size, cancer stage and poor differentiation of the tumor had adverse effect on both OS and DFS. Treatment modalities such as surgery, radiation, chemotherapy and hormonal therapies significantly improved both OS and DFS.
Title: Factors associated with mammographic density in Japanese women

Ken Uchida¹, Hitoshi Ohashi¹, Nogi Hiroko² and Rei Mimoto². ¹Shinjuku Medical Center, Shinjuku-ku, Tokyo, Japan and ²The Jikei University School of Medicine, Minato-ku, Tokyo, Japan.

Body: Background: Increased mammographic breast density decreases the sensitivity and the specificity of mammography screening and is associated with a significant risk factor for breast cancer. No studies were identified that examined the impact of supplemental screening on breast cancer recurrence rates or mortality for women with dense breasts. The aim of this study is to examine factors associating with breast density in Japanese women.

Data sources and methods: We used mammography check-up participants (n=5159, women aged 40 years or older) between Apr 2014 and Mar 2017 as our baseline data. Using a self-administered questionnaire, data of life style were collected. Logistic regression was used to estimate the odds of having dense breasts by age, body mass index (BMI), alcohol consumption, smoking, parity, menopausal status, dysmenorrhea, hormone replacement therapy (HRT), family history of breast cancer, physical exercise, fried foods intake, brightly colored vegetables intake, coffee intake, tea intake and bone mineral density. Breast densities were divided into four categories based on BI-RADS classification. BI-RADS 3 and 4 were defined as dense breast. All statistical tests were two-sided.

Results: Dense breast accounted for 62.3% of mammography screening participants. Dense breast at an early age was more frequent as 78.0% in the 40's. Alcohol intake (20 g or more a day, OR=1.62, 0.026) in post-menopausal women showed statistically significant positive interaction with dense breast. Weak positive interaction was seen in bone mineral density>80% (OR=0.63, 0.086, n=775). On the other, age (OR=0.97, <0.001), BMI (OR=0.78, <0.001), number of live birth (one; OR=0.77, <0.030, two or more; OR=0.37, <0.001) and post-menopause (OR=0.60, <0.001) showed statistically significant negative interactions with dense breast.

Conclusion: Dense breast accounted for 62.3% of all participants. Dense breast was more frequent at early age as 78.0% in their 40's. Alcohol consumption and bone mineral density in post-menopausal women were positive interaction with mammographic breast density. On the contrary, age, BMI, number of live birth and post-menopause were negative interaction with mammographic breast density.
Title: Treatment patterns for young women with HR+/HER2- metastatic breast cancer in the United States in the era of CDK 4/6 inhibitors

Harold J Burstein1, Erica L Mayer1, Angela DeMichele2, James Harnett3, Jack Mardekian3, Lynn McRoy3, Cynthia Huang Bartlett3, Maria Koehler3 and Mothaffar Fahed Rimawi4. 1Dana Farber Cancer Institute, Brigm and Women’s Hospital, Harvard Medical School, Boston, MA; 2Perelman School of Medicine University of Pennsylvania, Philadelphia, PA; 3Pfizer, Inc, New York, NY and 4Dan L Duncan Cancer Center at Baylor College of Medicine, Houston, TX.

Body: Background: NCCN guidelines recommend that premenopausal women with HR+/HER2- metastatic breast cancer (MBC) be rendered postmenopausal and then treated accordingly. After its approval in February 2015, the CDK4/6 inhibitor palbociclib (P), in combination with endocrine therapy (ET), has become a standard of care in the first-line or pretreated settings for women with HR+/HER2- MBC. Specialty pharmacy prescription data indicate that 12% of all women with HR+/HER2- MBC treated with P in the United States are younger than 50 years of age. We assessed the real world treatment patterns and outcomes before and after approval of P in women with HR+/HER2- MBC. We further sought to assess the impact of the NCCN guidelines for premenopausal women on treatment patterns and outcomes.

Methods: This retrospective cohort study utilized electronic health record (EHR) data from Flatiron Health (Fl) from 1/2012 through 4/2017 to evaluate patient characteristics and first-line ET treatment patterns among women with HR+/HER2- MBC prior to and after P approval. Menopausal status was defined by age (< 50 vs >50 yrs). Additional datasets of > 13,000 pts with MBC in the Truven Health MarketScan and Optum Clininformatics claims and Humedica EHR databases will be included to represent a more comprehensive dataset and evaluate clinical outcomes.

Results and Discussion: Initial results include 4,537 pts in the Fl database who initiated a first-line ET regimen. Overall, 30% of pts < 50 yrs used P compared to 29% of women age >50. Treatment patterns for initial endocrine therapy are shown in the table.

<table>
<thead>
<tr>
<th>Initial Endocrine Therapy</th>
<th>Women &lt;_50 yrs N (%)</th>
<th>women &gt; 50 yrs N(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%)</td>
<td>296 (100%)</td>
<td>273 (100%)</td>
</tr>
<tr>
<td>ET monotx +/- LHRH</td>
<td>296 (100%)</td>
<td>192 (70%)</td>
</tr>
<tr>
<td>TAM</td>
<td>108 (36%)</td>
<td>75 (27%)</td>
</tr>
<tr>
<td>AI</td>
<td>139 (47%)</td>
<td>80 (29%)</td>
</tr>
<tr>
<td>FUL</td>
<td>49 (17%)</td>
<td>37 (14%)</td>
</tr>
<tr>
<td>ET + P +/- LHRH</td>
<td>NA</td>
<td>81 (30%)</td>
</tr>
<tr>
<td>% of concurrent LHRH</td>
<td>77 (26%)</td>
<td>92 (34%)</td>
</tr>
</tbody>
</table>

Decreased use of tamoxifen as 1st line ET was observed in pts < 50 yrs over the observed time. 47% of young pts initiated endocrine based treatment with AI monotherapy in the pre-P era, consistent with the NCCN guidelines. About 26% (pre-P) and 34% (post-P) of pts ≤50 yrs initiated first ET with ovarian suppression in the analyzed treatment eras. The concurrent use of LHRH increased 8%.

Conclusions: The treatment paradigm for women with HR+/HER2- MBC has evolved over the last >5 years. Consistent with NCCN guidelines, more young pts are receiving ovarian suppression as part of initial therapy, and pts regardless of age are receiving treatment with P. There has been a related decrease in use of tamoxifen for younger pts and overall. These data illustrate rapid incorporation of palbociclib into standard care for US pts with HR+/HER2- MBC. Further treatment patterns and
therapy outcome data for these groups reflecting real world use ET regimens of over 17,000 pts in the combined cohort, will be reported.
Title: A randomized phase II trial of toremifene (120 mg) versus fulvestrant (500 mg) after prior non-steroidal aromatase inhibitor in postmenopausal women with hormone receptor-positive metastatic breast cancer (Hi-FAIR fx study)

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Body: Background: After the failure of a non-steroidal aromatase inhibitor (nsAI) for postmenopausal patients with advanced/metastatic breast cancer (BC), it is unclear which of the various kinds of endocrine monotherapy is the most appropriate. In a previous report it was found that toremifene 120 mg (TOR 120), a selective estrogen receptor modulator (SERM), was superior to steroidal AI in terms of progression-free survival after ns-AI in the Hi-FAIR ex trial. A phase II randomized trial of TOR 120 versus fulvestrant 500 mg (FUL 500), a selective estrogen receptor down regulator (SERD), was also conducted to select the most promising endocrine monotherapy after ns-AI in advanced/metastatic BC (Study registry number: UMIN000010087).

Patients and Methods: Postmenopausal women (n=106) with advanced/metastatic hormone-receptor positive BC from October 2011 to September 2014 were enrolled in this study. Fifty-three of the patients were randomly assigned to the TOR 120 (120 mg daily p.) group and 53 of the patients were randomly assigned to the FUL 500 group. In the FUL 500 group they were administered 500 mg of fulvestrant intramuscularly (im) on day 0, then 500 mg im on days 14 and 28 and every 28 days thereafter. If treatment failure occurred in either of the randomly assigned groups the patients were then removed and treated accordingly. A full analysis set was targeted for all cases that received the protocol treatment even once (TOR 120 (n=53) and FUL 500 (n=52)). The primary end point was the clinical benefit rate (CBR). The secondary end points were the objective response rate (ORR), progression-free survival (PFS), time to chemotherapy (TTCT), overall survival (OS), toxicity, and CBR, ORR and PFS after crossover of non-assigned treatment.

Results: A median follow up period of 30 months revealed that the CBR of FUL 500 (57.7%) tended to be superior to the CBR of TOR 120 (45.3%), the odds ratio (OR) was 1.70 (95% CI 0.74–3.62), and the median PFS was 7.8 months in the FUL 500 group and 5.8 months in the TOR 120 group. Moreover the hazard ratio (HR) was 0.79 (95% CI 0.52–1.21). However, there was no difference between the two groups in terms of ORR (17.7% and 15.1%, respectively), TTCT (13.3 months vs. 17.7 months, HR = 0.94 (95%CI 0.57 – 1.53)), and OS (33.4 months vs. not reached HR 1.29; 95% CI 0.80–2.09). At the cross-over phase, 33 and 24 patients after failure of assigned treatment were treated with FUL 500 and TOR 120, respectively. The CBR and PFS of FUL 500 after TOR 120 was better than that of TOR 120 after FUL 500 (CBR; 42.4% vs. 20.8%, OR = 0.33, 95%CI 0.09 – 1.11, median PFS; 6.2 months vs. 3.4 months; HR = 1.95, 95%CI 1.08–3.51). No difference between the two groups was observed in PFS from randomization to the end of the crossover phase. Moreover, there were few severe adverse events in either of the two groups.

Conclusions: FUL 500 used as a subsequent endocrine therapy for advanced/metastatic BC patients who failed ns-AI could potentially be more effective than TOR 120. However, the efficacy of SERM after failure of FUL 500 may be limited.
Title: The impact of dose delays and reductions on toxicity and progression free survival (PFS) in patients receiving palbociclib

Katherine K Clifton, Jaime Kimmel, Min Yi, Barnett Chad, Jennifer Litton, Tripathy Debu and Karuturi Meghan. MD Anderson Cancer Center, Houston, TX.

Body: Background: Despite the high rates of neutropenia observed in the PALOMA studies, the incidence of neutropenic fevers remained low. The safety analysis from the PALOMA-3 trial showed no difference in PFS among pts who had dose reductions or delays secondary to neutropenia. We conducted a retrospective study to analyze the impact of dose delays and reductions on toxicity and progression free survival (PFS) in pts receiving palbociclib as standard of care.

Methods: Pts with metastatic ER positive breast cancer receiving palbociclib in any line of therapy were identified from a cohort at MD Anderson Cancer Center. Clinical, demographic, baseline labs, comorbidities and recurrence data were collected. Dose delays, dose reductions, and toxicities were recorded up to the first 6 cycles of palbociclib. Early dose delays and reductions were defined as events occurring during the first 2 cycles of palbociclib while late events were defined as cycles 3-6. Data was analyzed using Fischer’s exact test for categorized variables and T test/Wilcoxon rank-sum test for continuous variables. PFS was analyzed using the Kaplan Meier method and Cox model was used to analyze factors associated with PFS.

Results: 344 pts who met eligibility criteria were included in the analysis. Pts receiving palbociclib on clinical trial were excluded. 109 (31.6%) pts received dose reductions and 153 (44.4%) experienced dose delays. The rate of neutropenic fever was low, occurring in 2.3% of all pts. There was a significant association between pts experiencing dose reductions and Hispanic race, baseline ANC, history of adjuvant endocrine therapy, adjuvant radiation therapy (XRT), and heart disease. History of adjuvant XRT, baseline ANC, and heart disease were associated with dose delays. Toxicities, including neutropenic fever, infections requiring antibiotics, and hospitalizations, were associated with dose reductions and dose delays. Median PFS for the cohort was 263.5 days. There was no significant association between early dose reductions or delays with PFS. Pts experiencing late dose delays (hazard ratio [HR], 0.4, P=0.0001) and reductions (HR, 0.4, P=0.0005) had a significantly longer PFS. Median PFS for pts without late dose delays was 228 days compared to 313.5 days for pts with late dose delays. Median PFS for pts without late dose reductions was 246 days compared to 305.5 days for pts with late dose reductions. In the multivariable analysis, liver metastasis, metastatic line, and higher tumor grade were associated with worse PFS. Pts receiving palbociclib and fulvestrant were found to have worse PFS than pts receiving palbociclib and letrozole.

Conclusions: Similar to the PALOMA trials, this study found that while the rate of toxicities such as neutropenic fever were low, dose reductions and delays were common. In pts receiving palbociclib as standard of care, pts with late dose reductions and delays had a longer PFS than those without dose reductions and delays. It is reassuring that the PFS was not negatively affected in pts with dose reductions and delays. As use of palbociclib as standard of care becomes more common, further larger retrospective studies are warranted to examine the impact of dose delays and reductions.
Title: Phase I/II trial of palbociclib in combination with bicalutamide for the treatment of androgen receptor (AR)+ metastatic breast cancer (MBC)

Ayca Gucalp¹, Marcia Edelweiss¹, Sujata Patil¹, Mrinal M Gounder¹, Kimberly N Feigin¹, Adriana Corben², Artavazd Arumov¹ and Tiffany A Traina¹. ¹Memorial Sloan Kettering Cancer Center and ²Icahn School of Medicine at Mount Sinai.

Body: Background: The androgen-signaling pathway plays a role in breast cancer (BC) pathogenesis and emerging evidence suggests the androgen receptor (AR) is a therapeutic target. TBCRC011 established safety and efficacy of inhibiting AR with bicalutamide (B) in patients (pts) with androgen AR+/ER/PgR- metastatic BC (MBC). Treatment with B at 150 mg oral daily demonstrated a clinical benefit rate (CBR) of 19% in this population. The rationale for combining palbociclib (P) with AR blockade stems from prior work in ER+ MBC. P is an oral, selective inhibitor of CDK4/6 activity which significantly improved median progression-free survival (PFS) in combination with letrozole compared to letrozole monotherapy for the treatment of postmenopausal pts with ER+ MBC in the first-line setting. Consistent with preclinical data, P has been shown to reduce growth of AR+ ER/PgR- MDA-MB-453 breast cancer cells via reduced Rb phosphorylation. It has been shown that AR+ TNBC expresses a luminal profile and has intact Rb protein, the target of palbociclib activity. Therefore, we hypothesize that P will increase the efficacy of B in pts with metastatic AR+ TNBC. NCT02605486.

Methods: Pts with AR+ (IHC ≥ 1%)/ER any/HER2(-) MBC on central review at MSK were eligible if met following criteria: ECOG ≤ 2, postmenopausal, no limit to prior regimens. Pts with ER+ BC must have had 1 prior endocrine therapy. Treatment: B orally daily and P orally daily 3 weeks on 1 week off. DLT period = 28 days. Pts are evaluated for toxicity every 2-4 weeks and for response every 8 weeks. Phase I standard 3+3 design with 3 dose escalations. The primary objective of the phase I portion of the study is to determine the recommended phase II dose of P in combination with B. Plasma for pharmacokinetics (PK) was collected throughout the study.

Results: As of 5/11/17, 15 pts with AR+ MBC were enrolled to the phase I portion. Target accrual has been met for the phase I and has since closed. No DLTs were reported in any of the dose escalation cohorts. The maximum tolerated dose was determined to be B 150 mg daily and P 125 mg daily for 21 days in a 28 day cycle. Treatment has been well tolerated with no related Grade 4 or 5 adverse events (AEs). The most common grade 3 significant AEs were hematologic including neutropenia n=5, leukopenia n= 4, and lymphocytopenia n=3 and thought to be related to P. One Grade 4 hypercalcemia led to hospitalization and was deemed related to disease progression. Median time on study was 8 weeks (2-47 weeks). One patient from the phase I remains on study. PK analysis is ongoing and will be presented, including drug-drug interaction data.

Conclusions: The combination of B+P has been well tolerated with no unexpected toxicity observed. Updated safety, response, and PK data will be presented. Enrollment on phase II is ongoing.
Title: Everolimus-exemestane (EE) vs palbociclib-fulvestrant (PF) or abemaciclib-fulvestrant (AF) or everolimus-fulvestrant (EF) in the treatment of metastatic HR+, HER2- metastatic breast cancer and prior aromatase inhibitors treatment. An indirect comparison with network meta-analysis

Lorenzo Gianni, Valentina Arcangeli, Caterina Gianni, Lucia Stocchi, Lorenzo Menghini, Domenico Samorani, Claudio Ridolfi, Tamburini Emiliano and Davide Tassinari. 1Infermi Hospital, AUSL Romagna, Rimini, RN, Italy; 2Servizio di Diagnostica Senologica e Prevenzione, Infermi Hospital, AUSL Romagna, Rimini, RN, Italy and 3UO General Surgery, Franchini Hospital, AUSL Romagna, Santarcangelo di Romagna, RN, Italy.

Body: Background. Treatment options for patients with hormone receptor positive metastatic breast cancer (MBC) and prior treatment with aromatase inhibitors (AIs) include EE or PF. To date no direct comparison has been presented between EE and Fulvestrant plus Cyclin-dependent Kinase 4/6 (CDK4/6) inhibitors in this setting. The objective of this study was to compare the efficacy of EE to PF or AF or EF in the treatment of metastatic HR+, HER2- breast cancer pre-treated with AIs.

Methods. An indirect comparison with a network meta-analysis comparing EE with PF or AF or EF in the treatment of metastatic HR+, HER2- breast cancer pre-treated with AIs was performed. The Progression-Free-Survival (PFS) was the primary end point of all our indirect comparisons. Efficacy data were expressed as Hazard Ratio (HR) and 95% Confidence Interval (95CI), assuming an α-error of 5% as index of statistical significance.

Results. All the data of the BOLERO-2 trial, the Bachelot et al network meta-analysis (Breast Cancer Treat Rep 2014), the Paloma-3, the Monarch-2, and the prECOG trials were analyzed and indirectly compared in a network meta-analysis. 6 orders of comparison were performed: AF vs PL, PF vs EE, AF vs EE, EF vs AF, EF vs PF and EF vs EE. The pooled HR and 95%CI are reported in table1.

Table 1: difference in PFS between comparators

<table>
<thead>
<tr>
<th>Comparisons</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF vs PF</td>
<td>1.202 (0.87-1.661) p = 0.265</td>
</tr>
<tr>
<td>PF vs EE</td>
<td>1.674 (0.995-2.818) p = 0.89</td>
</tr>
<tr>
<td>AF vs EE</td>
<td>2.012 (1.09-3.714) p = 0.025</td>
</tr>
<tr>
<td>EF vs AF</td>
<td>1.085 (0.681-1.728) p = 0.731</td>
</tr>
<tr>
<td>EF vs PF</td>
<td>1.304 (0.804-2.117) p = 0.283</td>
</tr>
<tr>
<td>EF vs EE</td>
<td>2.183 (1.072-4.444) p = 0.031</td>
</tr>
</tbody>
</table>

Conclusions. Till today EE and PF represent active and approved treatments for patients with metastatic HR+, HER2- breast cancer treated with AIs. The results of Monarch-2 study with AF vs Fulvestrant and prECOG study with FE vs Fulvestrant were recently presented and were included in this metaanalysis, while the results of Ribociclib and Fulvestrant vs Fulvestrant (Monaleesa-3 study) are not currently available.

These studies generally show that combination of hormone therapies with Everolimus or CDK4/6 inhibitors are better than hormone-therapy alone, however no direct comparisons between these treatment combinations exist in literature. The results of our indirect treatment comparisons suggests that EE is similar and, in some cases, it may be even better than other treatment options. The optimal treatment strategy and sequence for patient with MBC and prior treatment with AIs should be evaluated in clinical trials. Meanwhile these data could be considered together with safety and the economic profile to help physicians in daily clinical practice.
Title: A phase 1 study of KHK2375 (entinostat) as monotherapy and in combination with exemestane in Japanese patients with hormone receptor-positive, HER2-negative, advanced or recurrent breast cancer

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Body: Background: Patients (pts) with hormone receptor-positive (HR+) and non-life threatening advanced or metastatic breast cancer (BC) are usually treated with sequential endocrine therapies. Endocrine therapies are continued until tumor cells acquire resistance to them, following which pts are switched to cytotoxic chemotherapy. Entinostat (ENT) is an oral inhibitor of class I histone deacetylases (HDACs) and is expected to be used for endocrine therapy-resistant pts. The efficacy of ENT in combination with an aromatase inhibitor (AI) for HR+ BC was demonstrated in a previous randomized phase 2 study. Because of the lack of data on safety and pharmacokinetics (PK) in Japanese HR+ BC pts, we performed this dose escalation phase 1 study to investigate the safety of ENT monotherapy and combination therapy with exemestane (EXE) in postmenopausal women with advanced or recurrent HR+ BC. Secondary objectives were to assess PK and efficacy.

Methods: This study was based on a 3+3 dose escalation design. Postmenopausal women with advanced or recurrent HR+ HER2- BC previously treated with nonsteroidal AIs and with ECOG PS 0-1 were enrolled. The dose limiting toxicities (DLT) of ENT monotherapy (3 mg/qw, 5 mg/qw, or 10 mg/q2w) in Cohort 1-3 and those of ENT (5 mg/qw or 3 mg/qw) in combination with EXE 25 mg/qd in Cohort 4-5 were assessed for 7 and 28 days, respectively. Pts continued ENT (3 mg or 5 mg) in combination with EXE even after the DLT observation period until disease progression or discontinuation for other reasons. Adverse events (AEs) were graded per NCI-CTCAE version 4.03. Tumor response was evaluated by RECIST version 1.1 every 8 weeks. ENT concentration was measured intensively. Samples of peripheral blood mononuclear cells (PBMC) were collected to measure protein lysine hyperacetylation and for immune subset analysis. Optional tumor biopsies for biomarker assessment were collected before and during treatment.

Results: Twelve pts were enrolled and three each were assigned to Cohort 1-4 between Nov 2015 and Sept 2016. Neither DLT nor grade 3-5 AE occurred. As no DLT occurred in Cohort 4, Cohort 5 was omitted as originally planned. The drug-related AEs observed in ≥2 pts during the DLT observation period were grade 1-2 hypophosphatemia (1 pt each in Cohort 2, 3, and 4), grade 1 nausea (1 pt in Cohort 3 and 2 pts in Cohort 4), and grade 1-2 platelet count decreased (2 pts in Cohort 4). AUC₀-₁₆₈ increased in a dose proportional manner. As of May 2017, 4 pts continue to receive study treatment, including one treated for more than 18 months. Biomarker data including protein lysine hyperacetylation and immune subset in PBMC and results of paired biopsy samples will be reported.

Conclusions: This study showed the tolerability of the combination therapy of ENT 5 mg with EXE 25 mg in Japanese pts. There were no new safety concerns as compared to those reported previously. Following this result, a randomized phase 2 study for Japanese pts is planned.

Clinical trial information: NCT02623751.
2017 San Antonio Breast Cancer Symposium

**Publication Number:** P3-11-07

**Title:** Factors associated with first line chemotherapy use in patients with hormone receptor positive, HER2 negative metastatic breast cancer – data from the PRAEGNANT breast cancer registry

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**Body:**

**Background**

For breast cancer patients with metastases which are not life threatening national and international guidelines recommend the exhaustion of all antihormonal therapeutic options before recommending chemotherapy. In Germany up to now only everolimus was an additional option to overcome endocrine resistance. CDK4/6 inhibitors recently became available in Germany (Nov 2011). Aim of this analysis was the identification of predictors for a decision against an antihormonal treatment.

**Methods**

The PRAEGNANT metastatic breast cancer registry (NCT02338167) is a prospective registry for metastatic breast cancer patients. Besides biomarker research the description of real-world treatment data was one of the main study aims. This analysis was restricted to first line metastatic patients who were hormone receptor (HR) positive and HER2 negative. First, predictors were identified with a multiple logistic regression model. Then patients, who received chemo or not, were compared with regard to overall survival using Cox regression analysis with the predictors for chemotherapy from above and additionally chemo status (yes/no).

**Results**

A total of 389 HR-positive and HER2-negative patients with detailed treatment information were included during the 1st line therapy into PRAEGNANT. Of those 173 (44.5%) received a chemotherapy, 190 an antihormone therapy (AHT) (48.8%), and 26 (6.7%) everolimus+AHT. In the multiple logistic regression model, older patients, lower graded tumors, bone only disease and previous adjuvant chemotherapy were associated with a lower rate of first line chemotherapies. BMI and number of concomitant diseases had an influence with patients having a G1, G2 and G3 tumor receiving 1st line chemo in 28.0%, 38.4% and 63.2% of the cases respectively. Patients who received chemo seemed to have a worse overall survival than patients who did not receive chemo (adjusted HR 1.58; 95% CI, 0.89 to 2.18). However, this result was not significant (p = 0.12). Overall survival was primarily influenced by ECOG and location of metastasis.

**Conclusion**

The usage of chemotherapy can be predicted with age, metastasis pattern, grading and previous use of chemotherapy. However, we could not show that patients benefited from chemotherapy. On contrary, there was a tendency that patients treated with...
chemotherapy had poorer overall survival. Further studies with larger sample sizes are needed to confirm this claim.
Population characteristics and utilization patterns of patients treated with palbociclib over 2 years of post-approval


Background: Palbociclib (PAL) was the first-in-class cyclin dependent kinase (CDK) 4/6 inhibitor approved in the United States (US) in combination with an aromatase inhibitor [AI] or fulvestrant for the treatment of HR+/HER2- advanced/metastatic breast cancer (ABC/MBC) as initial or later-line endocrine therapy (ET). Over 60,000 patients have been treated in the US with PAL since its approval (02/2015). We described population characteristics and utilization patterns in patients who initiated treatment with PAL, using a real-world oncology electronic health record (EHR) database.

Methods: This was a retrospective observational study using de-identified Flatiron (FI) EHR data. As of study cutoff, the FI provider network comprises over 265 community cancer clinics and 3 academic cancer centers across 2500 clinicians and more than 1.5 million active cancer patients throughout the 50 states in the US. Adult MBC patients with a record of initiation with PAL on or after 02/03/2015 (drug approval) and prior to 03/31/2017 (study cutoff) were identified. The line of therapy (LOT) in the metastatic setting was assigned by evaluating systemic treatments (including chemotherapy) pre-and post-PAL initiation. The combination ET was defined as having a record of an ET within 28 days of the recorded order of PAL.

Results: Overall, 1871 patients were identified as having initiated PAL: 1841 (98.4%) are females and 30 (1.6%) are males. Of those females, 1057 (57.4%) received PAL with an AI and 752 (40.8%) received PAL with FUL. Mean follow-up from PAL initiation was 10.2 mos (SD: 6.7) and 36.8% were followed up for >12 mos. Mean age was 64.5 yrs (12.2% ≤50 yrs, 53.1% ≥65 yrs and 21.9% ≥75 yrs). Confirmed HR+/HER2- was observed in 85.1% of patients. Of patients with available ECOG-performance score (PS) at PAL initiation (n=1062), 82.1% had score 0/1, 14.6% had score 2, and 3.3% had score 3/4. Of patients with a known PAL starting dose (n=765), 90.6% initiated at 125mg, 6.7% at 100mg and 2.7% at 75mg. Among patients who initiated PAL with an AI, 45.7% initiated at LOT1, 23.8% at LOT2, 30.6% at LOT3+. Among patients who initiated PAL with FUL, 22.3% initiated at LOT1, 32.5% at LOT2, 45.2% at LOT3+. Among all patients who initiated PAL at LOT1 (regardless of combination partner; n=726), 65.7% used PAL with an AI. A total of 26.3% of patients received chemotherapy before PAL initiation among all females in the metastatic setting, and the most frequent ET regimen following PAL treatment is FUL alone (15.6%).

Conclusions: This study described the palbociclib population and utilization over 2+ years post-approval. Patients who initiated PAL represent the spectrum of those with HR+/HER2- MBC, including both females and males (1.6% of all PAL users). Among female patients, 12.2% were ≤50 yrs of age and 21.9% ≥75 yrs, and 17.9% had ECOG score ≥2 at initiation of PAL. Among all females treated with PAL in combination with an AI, more than 45% of patients initiated the regimen at the first line in the metastatic setting. Results with additional follow-up for the same cohort and data on real-world clinical outcomes (provided data become available) will be presented at the conference.
Title: 0.005% estriol vaginal gel in hormone receptor-positive postmenopausal women with early stage breast cancer in treatment with aromatase inhibitors (AIs) in the adjuvant setting. A phase II prospective, randomized, double-blind placebo-controlled study - “the Blissafe study”

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Body: BACKGROUND: 0.005% Estriol vaginal gel is a new formulation for the local treatment of postmenopausal vaginal atrophy which delivers an ultra-low dose of estriol (50mcg) per application. A study is proposed with the hypothesis that 0.005% Estriol vaginal gel is an efficacious and safe option to treat moderate to severe symptoms of vaginal atrophy caused by AIs without producing significant decline in gonadotropins or increase in systemic estrogens.

METHODS: 70 women with breast cancer receiving AIs and suffering from moderate to severe symptoms of vaginal atrophy were randomized to receive 1g of estriol gel or placebo gel (4:1) daily for 3 weeks and twice weekly up to 12 weeks. Preliminary data from an initial phase comprising 10 women treated for 3 weeks suggested that 0.005% Estriol vaginal gel did not have an influence on hypophyseal axis or estrogens. In the second study phase planned in 60 women, vaginal dryness and other symptoms and signs were analyzed with a 4-point severity scale (none, mild, moderate, severe) at baseline and at weeks 3 and 12. Estriol (E3), estradiol (E2) and estrone (E1) were analyzed by ultrasensitive LC-MS/MS assay (LOQ: 1pg/ml, 3pg/ml and 5pg/ml for E3, E2 and E1, respectively) at baseline and at weeks 1, 3, 8 and 12. FSH and LH were determined by Chemiluminiscency at the same timepoints and also at screening. Changes in vaginal parameters and hormonal levels were assessed. Adverse events were collected.

RESULTS: 61 women aged 59.2 (7.1) from 5 sites in Spain and Karolinska University Hospital in Sweden comprised the ITT analysis. At baseline, all women but one were treated with anastrozole or letrozole. One-third complained from moderate and two-thirds from severe vaginal dryness. 50 received estriol and 11 placebo. Women that received estriol slightly increased E3 levels [median (Q25-Q75)] to 3.9 (0.5-12.1), 1.9 (0.5-6.8), 0.5 (0.5-6.0) and 0.5 (0.5-7.3) pg/ml at w1, w3, w8 and w12 respectively. In these women E2 and E1 remained below LOQ in all samples but one at w12. Variation of FSH between baseline and w12 was not different from the variation of FSH before treatment (p=0.11 Wilcoxon), while small oscillations were observed between baseline and w1 and baseline and w3 as compared to FSH variation before treatment (p<0.05 Friedman, Dunn’s correction). A clinical superiority was observed at the end of treatment in women that received estriol in the improvement of their vaginal dryness, vaginal maturation value and score of vaginal signs vs those that received placebo ( p<0.01, p=0.01, p<0.01, respectively, Mann-Whitney-Wilcoxon). One serious adverse event emerged before the patient initiated treatment.

CONCLUSIONS: These data confirm the efficacy of 0.005% Estriol vaginal gel in women with breast cancer suffering from bothersome vaginal symptoms, with a transitory negligible absorption of estriol in initial weeks and a non-significant variation of FSH after 12 weeks of treatment. These findings provide confidence for the safe use of 0.005% Estriol vaginal gel in women with breast cancer with an indication for vaginal symptoms improvement.
Predictors of adherence to adjuvant endocrine therapy (ET) for early breast cancer (BC) in a prospective clinic-based cohort

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Body: Background: Adjuvant ET is associated with improved survival in women with hormone receptor-positive early BC. Nonetheless, more than a quarter of women are non-adherent or discontinue therapy early. We aimed to identify whether baseline characteristics and changes in weight and patient-reported outcomes (PRO) early during the course of ET are associated with medication adherence behavior (MAB) in a prospective cohort.

Methods: We enrolled women initiating or switching adjuvant ET for stage 0-III BC in a prospective clinic-based cohort. Participants completed PRO questionnaires at baseline, and 3, 6, and 12 months (mo) after initiating ET. PRO questionnaires included FACT-ES, the NIH PROMIS measures for pain interference, fatigue, depression, anxiety, physical function, and sleep disturbance, and the MOS Sexual Functioning Scale. MAB was assessed by the Medication Adherence Questionnaire (MAQ). MAB was defined as high (MAQ score=0), or medium/low (MAQ score>0). Questionnaires were administered through the PatientViewpoint web-based interface. We tested changes in mean PRO scores from baseline to follow-up time points with paired t-tests. We explored associations between baseline characteristics, and changes in weight and PRO at 6 mo with MAB at 12 mo using Fisher's exact test, Wilcoxon rank sum tests and t-tests. P-values <0.05 were considered significant.

Results: From March 2012 to December 2016, 336 women enrolled in the cohort. Mean age was 60 (range 26-90), 84% were Caucasian, and 67% were post-menopausal. Overall, 57% received an aromatase inhibitor, 43% received tamoxifen, and 28% received prior taxane chemotherapy. Median follow-up was 12 mo. At baseline, 61% were overweight/obese, and 21% gained >5% of baseline weight by 12 mo. Mean baseline and follow-up scores at 3, 6 and 12 mo were within 1 standard deviation of reference population means for all PRO measures. Compared to baseline, endocrine symptoms were increased at 3, 6 and 12 mo (p<0.05), while sexual function and depression did not differ between baseline and any follow-up time point (p>0.05). At 6 mo, anxiety was reduced, physical function was improved and pain impact was reduced compared to baseline (p<0.05). MAB was high for 71% of participants at 12 mo. Preliminary data demonstrate that, compared to those with high MAB at 12 mo, women with medium/low MAB at 12 mo took fewer concomitant medications at baseline, and had more improvement in anxiety and sexual function at 6 mo. MAB at 12 mo did not differ according to race, type of ET, baseline weight or PRO measures, or 6 mo change in weight or other PRO measures.

Conclusions: Early changes in anxiety and sexual function during the course of adjuvant ET and the number of baseline concomitant medications may separate women with subsequent high versus medium/low MAB risk. Weight loss interventions and symptom management are needed for women receiving adjuvant ET during the first year of treatment. Our data will be used to create a model to predict MAB for validation studies and as the basis to devise interventions to improve adherence to adjuvant ET.
2017 San Antonio Breast Cancer Symposium

Publication Number: P3-12-03

Title: Impact of genetic polymorphisms on plasma levels of tamoxifen and its metabolites and toxicity: 6-months results of the adjuvant breast cancer longitudinal PHACS study (NCT01127295)

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Body: Supported by a PHRC grant (#09-18-005)

Background: The role of CYP2D6 genetic polymorphisms and plasma levels of active metabolites of tamoxifen (TAM) on clinical response and occurrence of side effects remains controversial. We conducted a prospective, adjuvant, multicentre, 3-year follow-up study of breast cancer patients in order to evaluate the relationships between pharmacogenetics, pharmacokinetics and toxicity of TAM and its metabolites (n=879) or aromatase inhibitors (AI, n=1098). The present report focuses on the evaluation at 6 months after inclusion of 864 patients treated with 20 mg/day TAM. The clinical results and the AI PG/PK analyses are described elsewhere (abstracts #851544 and #851525).

Methods: Residual plasma concentrations for tamoxifen and its 6 major metabolites (endoxifen ENDO, 4-hydroxy-tamoxifen 4-OH-TAM, N-desmethyl TAM, TAM-N-oxyde, 4'-OH-TAM and Z'ENDO) at 6 months after start of treatment were measured by UPLC-MS/MS in 789 patients. Nine patients with TAM concentrations below the limit of quantification were excluded for non-compliance. SNP genotyping of 95 selected SNPs was performed on the Biomark (Fluidigm) in a microfluidic multiplex 96 dynamic array chip with Taqman assays and was available for 857 patients. Patients were classified according to their CYP2D6 metaboliser status (MS) (PM, IM, EM and UM) based on presence of functional, decreased function or no functional alleles (*4, *6, *7, *9, *10, *17, *41) and number of CYP2D6 copies (*5 or duplication). Metabolic ratios (MR) were calculated for TAM/4-OH-TAM, TAM/N-desmethyl tamoxifen (NDT), NDT/ENDO and 4-OH-TAM/ENDO. Anti-estrogenic activity score (AAS) was calculated according to a recently proposed algorithm (De Vries Schultink et al., Breast Cancer Res Treat. 2017). Toxicity was measured as a binary outcome (first occurrence or worsening of hot flushes, fatigue, depression, pain, arthralgia, vaginal dryness). All genetic associations were adjusted for multiple testing.

Results: ENDO concentration and AAS increased significantly with CYP2D6 MS (p<0.001). The presence of a CYP3A4*22 allele was significantly associated with endoxifen concentrations; this association remained significant after adjusting for CYP2D6 MS. TAM/4-OH-TAM MR was significantly influenced by the presence of CYP3A4*22, CYP2C19*2 and *17, and CYP2D6 status. The percentage of patients having an AAS>=1798 (i.e., threshold previously associated with recurrence-free survival RFS by De Vries et al. 2017) was 6%, 50%, 84% and 91% of patients respectively classified as PM, IM, EM and UM. Side effects were not significantly associated with higher levels of TAM metabolites concentrations. After correction for multiple testing, SNPs or CYP2D6 MS were not significantly associated with occurrence or worsening of adverse events, premature treatment discontinuations or switch due to toxicity within the first 6 months.

Conclusions: In this large prospective study, we quantified the impact of PG on TAM PK and AAS, previously shown to predict RFS. Although the toxicity observed after 6 months of TAM does not seem correlated with PK or PG, these relationships need to be re-evaluated during the 3-year follow-up.
Title: Efficacy of extended adjuvant aromatase inhibitors in subgroups of women with early breast cancer

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Body: Background: Randomized trials (RCTs) have reported improvements in breast cancer outcomes from extending treatment with aromatase inhibitors (AIs) beyond the initial 5 years after diagnosis. It is uncertain whether this effect is consistent in different subgroups.

Methods: We identified RCTs that compared extended AIs to placebo or no treatment using a systematic search of MEDLINE. The search was supplemented by a review of abstracts from the American Society of Clinical Oncology and San Antonio Breast Cancer Symposium meetings between 2013 and 2016. Hazard ratios (HRs) and 95% confidence intervals (CI) for disease-free survival (DFS) were extracted or estimated from forest plots and included in a meta-analysis using generic inverse variance and random effects modelling. Pre-specified subgroups included age (<60 ± 5 years vs. ≥60 ± 5 years), tumor size (≤2 cm vs. >2 cm), nodal status (positive vs. negative), hormone receptor status (estrogen [ER] and progesterone receptor [PR] positive vs. ER or PR positive) and administration of adjuvant chemotherapy (yes vs. no).

Results: Seven trials comprising 16,349 patients were analyzed. Studies designs and prior endocrine therapy are shown in Table 1.

Table 1: Characteristics of included studies.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Treatment Arms</th>
<th>Sample size</th>
<th>Prior endocrine treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCSG 6a</td>
<td>Anastrozole 3 years vs. none</td>
<td>387/ 469</td>
<td>Tamoxifen± aminoglutethimide: 100%, 5 years</td>
</tr>
<tr>
<td>MA 17</td>
<td>Letrozole 5 years vs. placebo</td>
<td>2572/ 2577</td>
<td>Tamoxifen: 100%, −5 years</td>
</tr>
<tr>
<td>NSABP B-33</td>
<td>Exemestane 5 years vs. placebo</td>
<td>783/ 779</td>
<td>Tamoxifen: 100%, −5 years</td>
</tr>
<tr>
<td>Dutch DATA</td>
<td>Anastrozole 6 years vs.,</td>
<td>827/ 833</td>
<td>Tamoxifen: 100%, 2-3 years</td>
</tr>
<tr>
<td>IDEAL</td>
<td>Anastrozole 5 years vs. letrozole 2.5 years</td>
<td>903/ 898</td>
<td>Any endocrine treatment (tamoxifen/AIs/sequence of tamoxifen+ AIs): 100%, 5 years</td>
</tr>
<tr>
<td>MA.17R</td>
<td>Letrozole 5 years vs. placebo</td>
<td>959/ 959</td>
<td>Als: 100%, −5 years Prior tamoxifen: 79.3%</td>
</tr>
<tr>
<td>NSABP B-42</td>
<td>Letrozole 5 years vs. placebo</td>
<td>1959/ 1964</td>
<td>Any endocrine treatment (AIs/sequence of tamoxifen+ AIs): 100%, 5 years</td>
</tr>
</tbody>
</table>

The pooled effect of prolonged treatment with AIs in different subgroups is shown in the Table 2.

Table 2: Intra-subgroup comparison of longer AIs treatment effect by subgroups

| Subgroup A | Subgroup B | HR (95% CI) Subgroup A | HR (95% CI) Subgroup B | P for difference |
|------------|------------|------------------------|------------------------|----------------|---|
| Age <60 ± 5 | Age ≥60 ± 5 | 0.83 (0.70-0.99) | 0.85 (0.74-0.97) | 0.64 |
| T >2 cm | T ≤2 cm | 0.77 (0.55-1.06) | 0.88 (0.68-1.13) | 0.44 |
Overall, the effect of prolonged AIs was similar in all subgroups. However, non-significantly greater effect sizes were seen in patient with larger tumors, nodal involvement, presence of both ER and PR expression and those treated with adjuvant chemotherapy.

Conclusions: Extended treatment with adjuvant AIs is associated with similar relative improvements in DFS in all subgroups analyzed. The greater effect size seen in node positive and large tumor subgroups and the higher baseline risk of recurrence will likely translate to a higher absolute benefit from extended AIs in these groups.
Serum levels of the active tamoxifen metabolite Z-4OHtam is predictive of long-term survival in luminal B subtype of breast cancer patients

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Body: Background

Tamoxifen (tam) is the main adjuvant endocrine treatment option in premenopausal breast cancer (BC) patients comprising luminal-like tumors. However, a significant proportion of tam-users will experience a relapse within 15 years of primary surgery. We postulate that some patients do not achieve the full clinical benefit of tam due to inter-individual differences in the metabolism of the drug and that the clinical relevance of this may be different between molecular subtypes of BC. Here, we have compared the prognostic value of threshold levels of active tam metabolites in PAM50 luminal (lum) A and B molecular subtypes.

Material and Methods

A number of 64 lum-like BC patients who were relapse-free 3 years after surgery, were retrospectively analyzed in the observational Oslo1 study. All patients received 20 mg tamoxifen daily for 5 years. Serum was obtained at the time of the 3 years follow-up. A sensitive and accurate LC-MS/MS method was developed and validated for the detection and quantification of tam and 9 metabolites in human serum. The median follow-up time from serum sampling to BC death or last follow-up was 13.9 years (0.6-16.5). Recurrence score and molecular subtype of the patients were determined on FFPE-tumor samples using the PAM50 classification algorithm.

Results

A linear trend was identified for the correlation between active metabolite Z-4OHtam and BCSS (p=0.021, HR=0.64, CI95=0.43–0.93). There was no linear association between the remaining metabolites and BC outcome. We further explored the possible association between survival and concentration thresholds for the active metabolites Z-4OHtam and Z-endoxifen and identified supervised cut off values representing low concentrations for Z-4OHtam (≤3.26 nM) and Z-endoxifen (≤9.00 nM). BC patients with low Z-4OHtam had a BCSS of 33.3% compared to 82.8% in patients with Z-4OHtam >3.26 nM (p<0.001, logrank; HR=6.83, CI 95=2.09-22.36). Lum status (A vs B; HR=5.50, CI95= 1.66-18.25) and Z-4OHtam concentration status (high vs low; HR=6.05, CI95=1.74-21.06) were the only factors left in the final multivariable model. A log-linear relationship between the ROR score and BCSS (p=0.002, HR=1.09, CI95=1.03–1.15) was identified after adjustment of clinically relevant variables and lum status was highly prognostic, (Lum A vs B; p=0.001, HR=5.2, CI=1.72-15.46). Therefore, we wanted to compare the prognostic value of the Z-4OHtam threshold in patients subgroups stratified by lum status. Low concentrations of Z-4OHtam were associated with poorer survival for patients in the lum B group only (HR=4.94, CI 95=1.16-21-02). For the lum A patients no significant association was found.

Discussion

Low levels (≤ 3.26 nM) of the active tam metabolite Z-4OHtam was associated with a poorer long-term outcome in tam-treated BC patients. However, when grouping patients according to the PAM50-based molecular subtype, this was only significant in patients belonging to the lum B subtype. Our results suggest that higher levels of active tam metabolites and thus better ER blockage are more important in the more aggressive lum B subtype.
2017 San Antonio Breast Cancer Symposium

Publication Number: P3-12-06

**Title:** Fertility concerns and their impact on hormonal therapy decisions in young breast cancer survivors

Philip D Poorvu¹, Kathryn J Ruddy¹, Shari I Gelber¹, Rulla M Tamimi², Jeffrey Peppercomb³, Lidia Schapira⁴, Virginia F Borges⁵, Steven E Come⁶, Ann H Partridge¹ and Shoshana M Rosenberg¹. ¹Dana-Farber Cancer Institute, Boston, MA; ²Brigham and Women’s Hospital, Boston, MA; ³Massachusetts General Hospital, Boston, MA; ⁴Stanford University, Stanford, CA; ⁵University of Colorado Cancer Center, Aurora, CO, United Arab Emirates and ⁶Beth Israel Deaconess Medical Center, Boston, MA.

**Body:**

**Background:** Fertility is a critical issue for young breast cancer (BC) survivors and can be diminished by adjuvant chemotherapy or by age-related decline in ovarian reserve over time. Little is known about how fertility concerns affect decision-making and persistence with endocrine therapy (ET) given the standard 5-10 year duration of therapy during which pregnancy is contraindicated.

**Methods:** As part of a multi-center, prospective cohort study enrolling women with newly diagnosed (dx) BC at age ≤40 years between 2006-2016, we identified participants with HR+, Stage I-III BC, without documented recurrence and with at least 3 years of follow-up. Participants completed serial surveys that include questions about socio-demographics, fertility issues and outcomes, treatment, and decision-making. ET use and pregnancy outcomes were evaluated up to 5 years post-dx (mean follow-up: 4.4 years). We used t-tests and chi-square tests to evaluate differences between women who indicated at least once in the first 2 years following diagnosis that fertility concerns affected their ET decisions and those who did not, and multi-variable logistic regression to identify factors independently associated (p≤0.05) with indicating ET decisions were affected by fertility concerns.

**Results:** Among 479 women included in this analysis, 33% (156/479) indicated that fertility concerns affected their decision regarding hormonal therapy – by choosing to defer treatment, stop early, or indicating that they may stop early or interrupt at a future time. Among these women, 44% (67/156) did not initiate or stopped ET (at least temporarily) vs. 21% (68/323) among women who did not indicate that fertility concerns affected their decision (p<0.0001). Among the 67 women with fertility concerns who did not initiate/discontinued ET, 29 (43%) subsequently reported a pregnancy within 5 years of dx. Women who were younger at dx, not partnered, nulliparous, and those who had a pre-treatment discussion about fertility with a provider were more likely to indicate that fertility concerns affected their ET decision (Table). In multi-variable analyses, only no or low parity remained significant: no children at diagnosis vs. ≥2 OR 9.86, 95% CI: 5.19-18.75, 1 child at diagnosis vs. ≥2: OR 6.28, 95% CI: 3.18-12.39.

**Conclusion:** Concern about fertility is a contributor to ET decisions among a significant number of young women with HR+ BC. Ongoing research, including the POSITIVE trial (NCT 02308085), an international study that is exploring the safety and feasibility of interrupting ET for pregnancy after HR+ BC, will provide much needed evidence that will help inform and guide both patients and providers as they make fertility and treatment decisions.

<table>
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<td>2</td>
<td>69 (44)</td>
<td>146 (45)</td>
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</tr>
<tr>
<td>3</td>
<td>15 (10)</td>
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<td>1</td>
<td>31 (21)</td>
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<tr>
<td>≥ 2 children</td>
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<td><strong>Pre-treatment fertility discussion</strong></td>
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<tr>
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<td>127 (88)</td>
<td>225 (73)</td>
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<tr>
<td>No</td>
<td>17 (12)</td>
<td>84 (27)</td>
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Title: Pharmacogenetic determinants of aromatase inhibitors pharmacokinetics and side effects: 6-month results of the adjuvant breast cancer longitudinal PHACS study (NCT01127295)

Fabienne Thomas1,2, Pierre Marquet3, Frédéric Pinguet4, Melanie White-Koning2, Jacques Robert5, Naïma Tafzi3, Isabelle Solassol4, Raymond Despax6, Nadia Levasseur7, Stephen Ellis8, Angélique Massoubre1, Litaty Mbatchi9, Valérie Le Morvan5, Henri Roché1, Étienne Chatelut1,2 and Alexandre Evrard9. 1Institut Claudius Regaud, IUCT-O, Toulouse, France; 2CRCT, Inserm, Univ Toulouse, Toulouse, France; 3CHU Limoges, Limoges, France; 4ICRM, Montpellier, France; 5Institut Bergonié, Bordeaux, France; 6Clinique Pasteur, Toulouse, France; 7CH Cahors, Cahors, France; 8Centre Catalan d’Oncologie, Perpignan, France and 9CHU Carémeaux, Nîmes, France.

Body: Supported by a PHRC grant (#09-18-005)

Background: Recent literature has suggested that germline genetic variants of drug-metabolizing enzymes or CYP19A1 (coding for aromatase) may be involved in the systemic aromatase inhibitors (AI) concentrations or the occurrence of side effects (Hertz et al. Pharmacogenomics 2017). A prospective multicentre 3-year follow-up study was carried out to investigate the relationships between pharmacogenetics (PG), pharmacokinetics (PK) and toxicity in breast cancer patients treated with adjuvant AI (n=1098) or tamoxifen (n=879). The clinical results and the tamoxifen PG/PK analyses are described elsewhere (abstracts #851544 and #850248).

Methods: SNP genotyping of 95 SNPs was performed on the Biomark (Fluidigm) with Taqman assays and was available for 373, 515 and 151 patients treated with anastrozole (ANA), letrozole (LETRO) and exemestane (EXE) respectively. CYP2A6 metaboliser status (MS) (poor, intermediate or normal) was determined based on alleles function (*1, *9, *2) and number of CYP2A6 copies. Trough plasma concentrations of each drug were determined 6 months after the start of the study by UPLC-MS/MS and were available for 342, 463 and 130 patients of the ANA, LETRO and EXE arms. Patients with AI concentrations below the limit of quantification were excluded for non-compliance (9 patients for ANA, 8 patients for LETRO and 7 patients for EXE). Toxicity was measured as a binary outcome (occurrence or worsening of hot flushes, fatigue, pain, arthralgia, vaginal dryness). All genetic associations were adjusted for multiple testing.

Results: ANA concentration was significantly higher in patients experiencing pain (p=0.025) and was associated with rs28365063 (UGT2B7 g.372A>G).

LETRO concentrations were strongly associated with CYP2A6 metabolizer status (p=0.0001) but did not differ in patients with or without toxicity.

In the EXE arm, patients with hot flushes or arthralgia had a significantly lower level of exemestane (p= 0.0002 and p=0.023 respectively) but since the metabolism of EXE leads to active 17-hydroxexemestane, we can hypothesize that the lower EXE concentration is an indirect reflection of the metabolite formation. A SNP (rs2307424) in NR1I3 gene (coding for the constitutive androstane receptor CAR) was associated with EXE concentrations. CAR has been shown to regulate CYP2B6, which is involved in the formation of 6-hydroxy-methyl-exemestane (inactive metabolite).

Regarding the relationships between PG and toxicity, in the ANA arm, 3 SNPs of CYP19A1 gene tended to be associated with hot flushes worsening (rs934635) and arthralgia (rs10046 and rs2304463) but did not remain significant after multiple tests correction. In the EXE arm, several SNPs in NR1I3 gene were associated with EXE concentrations. CAR has been shown to regulate CYP2B6, which is involved in the formation of 6-hydroxy-methyl-exemestane (inactive metabolite).

Conclusions: Our study confirms the predominant role of CYP2A6 in LETRO PK. To our knowledge, this is the first study to report on the role of UGT2B7 rs28365063 in ANA and NR1I3 in EXE PK and side effects. These relationships need to be re-evaluated with the drug concentrations obtained during the 3-year follow-up.
2017 San Antonio Breast Cancer Symposium

Publication Number: P3-12-08

Title: Evaluation of treatment compliance during extended endocrine therapy; secondary analysis of the IDEAL trial

Erik J Blok\textsuperscript{1,2}, Judith R Kroep\textsuperscript{2}, Elma Meershoek-Klein Kranenbarg\textsuperscript{1}, Marjolijn Duijm-de Carpentier\textsuperscript{1}, Johan WR Nortier\textsuperscript{2}, Emiel JTh Rutgers\textsuperscript{3} and Cornelis JH van de Velde\textsuperscript{1}. \textsuperscript{1}Leiden University Medical Center, Leiden, Netherlands; \textsuperscript{2}Leiden University Medical Center, Leiden, Netherlands and \textsuperscript{3}Netherlands Cancer Institute, Amsterdam, Netherlands.

Body: In the first clinical trial reports about extended endocrine therapy in early breast cancer, treatment compliance appeared as a major concern. Earlier, it was shown in the IDEAL trial that approximately 35% of all patients stopped therapy before the allocated time. This additional study was conducted to evaluate the factors contributing to early treatment discontinuation.

Methods: In the IDEAL trial, a total of 1824 patients were randomized between either 2.5 or 5 years of extended letrozole, after 5 years of any adjuvant endocrine therapy. Only eligible patients who started therapy were included in the analysis. Adverse events were collected until 30 days after last treatment dose Reasons for ending therapy were collected prospectively at the time of treatment discontinuation.

Results: The majority of early treatment discontinuation was caused by adverse events (AEs) (n=372, 20.4% of all patients, 58% of all early treatment discontinuations). The most frequently reported AEs associated to treatment discontinuation were arthralgia (n=71, 9.9% of AEs associated treatment discontinuation), fatigue (n=48, 6.7%), depression (n=47, 6.5%), hot flashes (n=47, 6.5%) and alopecia (n=39, 5.4%). Of all AEs associated to early discontinuation, 86% was grade 1 or 2 (table 1). All grade 5 events were not associated to therapy.

Table 1 - Overview of adverse events most frequently associated to early treatment discontinuation

<table>
<thead>
<tr>
<th></th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
<th>Grade 5</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthralgia</td>
<td>22</td>
<td>36</td>
<td>12</td>
<td>1</td>
<td>0</td>
<td>71</td>
</tr>
<tr>
<td>Fatigue</td>
<td>19</td>
<td>26</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>48</td>
</tr>
<tr>
<td>Depression</td>
<td>20</td>
<td>21</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>47</td>
</tr>
<tr>
<td>Hot flashes</td>
<td>16</td>
<td>20</td>
<td>9</td>
<td>2</td>
<td>0</td>
<td>47</td>
</tr>
<tr>
<td>Alopecia</td>
<td>28</td>
<td>7</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>39</td>
</tr>
<tr>
<td>Total (all AEs)</td>
<td>302</td>
<td>316</td>
<td>80</td>
<td>13</td>
<td>6</td>
<td>720</td>
</tr>
</tbody>
</table>

Furthermore, the influence of previous type of adjuvant endocrine therapy was evaluated. Of all patients initially treated with 5 years of tamoxifen, 29% stopped due to an AE. In contrast, patients who were treated with aromatase inhibitors during the first 5 years, either with monotherapy or after 2-3 years of tamoxifen, stopped due to AEs in 22% and 18% respectively (Pearson Chi-square p-value 0.001). The average number of AEs per patient per previous treatment group was 2.27 for tamoxifen monotherapy, 2.03 for AI monotherapy and 1.73 in the sequential group. Corrected for the number of AEs in each group, patients pre-treated with 5 years of tamoxifen had a chance of treatment discontinuation of 12.7% per AE, compared to 10.8% and 10.4% for AI monotherapy and sequential therapy respectively. Additionally, of patients that completed regular adjuvant therapy between 1 and 2 years before randomization, 34% stopped due to adverse events. In contrast, of patients that completed therapy within 6 months before randomization stopped in 19% of all cases (Pearson Chi-square p-value <0.001).

Conclusion: We have shown that adverse events are an important factor in early treatment discontinuation. Furthermore, the relation between adverse events and early discontinuation is influenced by the type of earlier therapy, with the highest rate of discontinuation for AI-naïve patients. This suggests that after 5 years of tamoxifen, patients are more inclined to stop therapy when encountering new AI-related adverse events compared to patients who were pre-treated with an AI.
2017 San Antonio Breast Cancer Symposium

Publication Number: P3-12-09

Title: CYP2D6*10 genotype was associated with worse outcome of premenopausal breast cancer patients receiving adjuvant tamoxifen but not toremifene: A single institution experience

Bo Lan1, Fei Ma1, Ying Fan1, Xiaoyu Zhai1 and Binghe Xu1. 1National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China.

Body: Background: Tamoxifen (TAM), a selective estrogen receptor modulator (SERM), is the most widely used adjuvant endocrine therapy for premenopausal breast cancer patients. Cytochrome P-450 (CYP450) enzyme, CYP2D6, is involved in the conversion of tamoxifen to endoxifen which is one of the main active metabolites of tamoxifen in vivo. Variants of CYP2D6 gene may result in a decreased enzyme activity and lead to poor prognosis of the patients. Different from caucasians, the most common polymorphism among Chinese women is allelic variant *10, which generates a 188 C to T transition, resulting in a lower activity of the enzyme. Based on some retrospective studies, tamoxifen-treated patients with the CYP2D6*10 T/T genotype have a worse clinical outcome. Toremifene (TOR), another kind of SERM is not metabolized by CYP2D6 enzyme thus may not be influenced by its polymorphism. We conducted this study to validate the association between CYP2D6*10 genotype and the outcomes of patients receiving TAM and TOR respectively.

Methods: A total of 276 patients with primary early-stage ER-positive breast cancer received adjuvant tamoxifen (n=169) or toremifene (n=107) therapy at National Cancer Center from 2004-2012 were analyzed. All patients had received 5-year endocrine therapy after completion of surgery. TaqMan SNP genotyping assays was performed on CYP2D6*10 from blood samples. The association of CYP2D6 *10 genotype with disease free survival (DFS) and clinicopathological characteristics was analyzed in patients receiving tamoxifen and toremifene.

Results: 32.6% (90 of 276) of the patients were homozygous for wild-type C/C genotype, 47.1% (130 of 276) were heterozygous for C/T genotype, and 20.3% (56 of 276) were homozygous for variant T/T genotype. The frequency of CYP2D6 *10 allele in our study was 43.8%. The 5-year DFS rate for tamoxifen and toremifene treatment group were 81.6% and 83.2% respectively. There was no significant difference of DFS between the two groups (P=0.274). Among 169 patients in tamoxifen group, 5-year DFS rate was considerably lower in patients with homozygous variant T/T genotype than those with wild-type C/C or C/T genotype (73.4% versus 83.2%, P=0.004). And the T/T genotype was found to be a significant prognostic marker for DFS in multivariate analysis (hazard ratio = 4.7; P<0.001) after adjusting for patient’s characteristics mentioned above. For the toremifene group, there was no difference of DFS between T/T genotype and the others (P=0.332). For all the 56 homozygous variant T/T genotype patients, patients receiving toremifene treatment had a much higher 5-year DFS rate than those receiving tamoxifen but unfortunately it was not statistically significant (90.0% versus 73.4%, P=0.192).

Conclusions: About one fifth of Chinese breast cancer patients had homozygous T/T genotype which might get less benefit from TAM adjuvant treatment. Toremifene may be a better option for this kind of patients. Further large-scale prospective clinical studies are warranted to validate this concept.
First 6-month report of the longitudinal PHACS study (Pharmacology and Hormonotherapy (HT) for Adjuvant breast Cancer (BC) Study, NCT01127295)


Institut Claudius Regaud, IUCT-Oncopole, Toulouse, Cedex 9, France, Metropolitan; CHU Limoges, Limoges, France, Metropolitan; Institut Bergonié, Bordeaux, France, Metropolitan; Institut de Cancérologie de Montpellier, Montpellier, France, Metropolitan; Clinique St Jean du Languedoc, Toulouse, France, Metropolitan; Centre Hospitalier, Brive, France, Metropolitan; Polyclinic Bordeaux Nord Aquitaine, Bordeaux, France, Metropolitan; Clinique Pasteur, Toulouse, France, Metropolitan; CHU Carémeau, Nîmes, France, Metropolitan; Clinique Tivoli, Bordeaux, France, Metropolitan; Centre de Recherche en Cancérologie de Toulouse, INSERM, Toulouse, France, Metropolitan.

Body: Background: BC is a hormone-dependent disease for 75% of pts. HT is used in both adjuvant and metastatic settings for hormone-receptor (HR) positive tumors. In adjuvant situation, a 5-year HT period at least is recommended. Side-effects (SE) frequently alter quality of life and compliance, reducing the well-known benefits in risks of relapses and specific deaths. Underlying mechanisms are well understood for estrogen deprivation-induced events such as hot flashes, but little is known on arthralgia under aromatase inhibitors (AI). So, pharmacogenomics (PG), pharmacokinetics (PK), potential medications interactions are of value to explain individual drugs exposures, possible related side-effects and compliance to treatment.

Methods: We performed a prospective, multicenter, longitudinal study registering early clinical outcomes and SE during the first 3 years of adjuvant HT with tamoxifen (T) or AI. All tumors expressed at least one HR (>10%). The choice of HT molecule and one-drug or sequential treatment were left to the investigator. Pts were followed every 6 months with clinical examination by the referent oncologist and PK sampling each time. Biologic research consisted in PG investigations of different genes involved in the PK and pharmacodynamics of T and AI (95 SNPs) at baseline. SE, concurrent medications and compliance were registered by both the pts on a diary card and the physician. Evaluation was done only on new occurrence or increased grade of symptoms.

Results: This first report focuses on characteristics of the population and the results after the 6 first months of treatment. Between June 2010 and October 2014, 23 centers recruited 2000 pts. 23 were excluded leaving 1977 fully evaluable women; 879 (44%) started with T, 1098 (55%) with AI (554 letrozole (L), 390 anastrozole (A), 154 exemestane (E)). 56% of them had previously received chemotherapy, 96% radiotherapy and 8% trastuzumab. Main characteristics were well balanced between the 2 classes of drugs; T was given mainly for pre- or perimenopausal pts. Most frequent co-morbidities were hypertension (8% T, 31% AI) and dyslipidemia or diabetes (T 11%, AI 26%). To note, almost 30% of pts described arthralgias at entrance and 37% had hot flashes. At 6 months, 122 pts (6%; 43 T, 79 AI) had stopped treatment mainly for toxicity (11 T; 12 AI), progression or death (7 T; 4 IA), personal reasons (15 T; 37AI); 4 asked for changing T and 52 AI (equally for the 3 drugs). All these events were significantly more frequent for AI pts (p=0.042) and with E within the AI class (p<0.001). Main changes in onset or increased intensity of symptoms concerned hot flashes with all drugs (30%), asthenia (20%), insomnia (20%), weight gain (17%), arthralgias (15% for T, 30% for AI), thrombotic events (24 of which 11 with T). 3 grade3 SAE HT-related were reported.

Conclusions: These preliminary data on the first 6-months exposure to HT on adjuvant setting in the real-life confirm early rates of withdraws and toxicities. Longer follow-up and subsequent PK analysis should help to understand persistent side-effects and reasons for non-compliance to adjuvant HT.
Title: Disparities in adjuvant hormone adherence in breast cancer patients within a universal healthcare model

Zachary W Veitch1, Omar F Khan1, Derek Tilley2, Xanthoula Kostaras2, Karen King3, Sasha Lupichuk1 and Patricia Tang1. 1Tom Baker Cancer Centre - University of Calgary, Calgary, AB, Canada; 2Alberta Health Services - Cancer Control, Edmonton, AB, Canada and 3Cross Cancer Institute - University of Alberta, Edmonton, AB, Canada.

Body: Background: Patient adherence to adjuvant hormonal therapy for breast cancer (BC) is correlated with improved survival. Recent publications have demonstrated ethnic disparities in adjuvant hormone adherence (AHA) for privatized healthcare models. Objective: To identify disparities in AHA for BC patients within a universal healthcare system in Alberta, Canada. Methods: Patients diagnosed from 2007-2014 with stage I-III, ER+/HER2- BC receiving adjuvant FEC or DC chemotherapy and at least one month of adjuvant hormonal therapy in Alberta, Canada were retrospectively assessed. Hormone monotherapy (tamoxifen, AI), switch strategies (tam to AI), and treatment duration were collected. Compliance was assessed with central pharmacy data. Patient ethnicity was identified using patient first/last and parental last name via Onolytics® ethnographic software. Ethnicity was further verified using a centrally collected place of birth. Age, AJCC stage, psychiatric diagnoses (mood, bipolar), and comorbidity were collected. Log rank and Chi squared were used to assess difference between adjuvant hormonal therapy for variables at 1, 2, and 5 years. Log rank p-values at 2 years are reported. Results: A total of 2,399 ER+ patients were included for analysis. AHA was non-significant for ethnicity (p=0.797), comorbidity (p=0.623), psychiatric disorders (p=0.145), or elderly cohorts (p= 0.814). AHA was highest for planned hormonal switch strategies (p=0.004) compared to monotherapy.

Hormone Adherence Rates

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<tr>
<td>Middle Eastern/African</td>
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<td>Hispanic</td>
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<tr>
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<tr>
<td>&lt;65</td>
<td>2077</td>
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<td>&gt;65</td>
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Hormone Strategy

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<th>Strategy</th>
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<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monotherapy</td>
<td>1731</td>
<td>90.9%</td>
</tr>
<tr>
<td>Switch</td>
<td>687</td>
<td>94.2%</td>
</tr>
</tbody>
</table>

**Conclusion:** AHA is not dependent on ethnicity, age, comorbidity, or psychiatric diagnosis in a universal healthcare model. Conversely, higher rates of AHA are seen with planned switch strategy compared to monotherapy, contradictory to the BIG I-98 trial. Patients with higher stage, and thus higher risk of BC recurrence have increased adherence compared to their low risk counterparts. Reinforcement of AHA for low/moderate risk BC patients, in addition to tamoxifen to AI switch strategies may improve overall adherence.
**Title:** Serum estrogen-levels in women 48-56yrs with hormone receptor-positive breast cancer. Cross-Sectional study using an ultra-sensitive LC-MS/MS-method

Maxime Van Houdt¹, Patrick Neven¹, Lintermans Anneleen¹, Jans Ivo¹, Vanderschueren Dirk¹, Billen Jaak¹, Laenen Annouschka¹ and Wildiers Hans¹. ¹University Hospitals of Leuven, Leuven, Belgium.

**Background and Purpose:** Randomized clinical trials in early stage hormone receptor positive breast cancer (HR+BC), have shown that total estrogen suppression (TES) for 5 yrs or after 2-3 yrs of tamoxifen treatment improved outcome over 5 yrs of TAM monotherapy. This relative improvement is in absolute figures most visible in patients with a high relapse risk. To achieve TES, women need to be (rendered) menopausal and treated with an oral aromatase inhibitor (AI). We measured serum estrogens in women of peri-menopausal age with HR+BC starting or on anti-hormonal agents.

**Methods:** Serum estradiol (E2) and estrone (E1) were quantified using a sensitive liquid chromatography-tandem mass spectrometry method (LC-MS/MS; Pauwels et al. JCO 2013) in women 48-56 yrs old with HR+BC starting or on anti-hormonal therapy in a cross sectional observational monocentric study. We report the frequency that (and explore why) AI-users had estrogens above the lower limit of quantification (LLOQ). We measured estrogen levels in TAM users with > 3 months' amenorrhea by previous use of (neo)adjuvant chemotherapy. Finally, we studied the frequency of both estrogens or either alone were < LLOQ in non-users. Statistics are descriptive; biomarkers for estrogen levels were analyzed using linear mixed models.

**Results:** Estrogen levels were assessed in 566 samples from 401 women; 96 (126 samples) were on AI, 128 (182 samples) on TAM and 217 (258 samples) non-users. Some patients had more than one blood sample in different groups. 13.5% of AI users were above LLOQ for E2 and E1; 7.5% for E1 alone. Some were due to ovarian reactivation. An important proportion (18%) of 83 non-hysterectomized TAM users with amenorrhea had E2 levels >40ng/L; 9.1% and 28.2% respectively in patients who had and didn't have (neo)adjuvant chemotherapy. None of non-users were below LLOQ for E1, while 20.7% were below LLOQ for E2.

**Conclusions:** TES is not always achieved in AI-users; reasons why were multiple. Amenorrhea is no guarantee for TES in TAM users, as a significant proportion had high E2 levels even when previously exposed to (neo)adjuvant chemotherapy. Using LC-MS/MS, undetectable levels for E1 better reflect TES than below LLOQ for E2.
**Body: Background:** Atypical hyperplasia of the breast is a high-risk benign lesion that is found in approximately 10% of benign breast biopsies[1] and confers a risk for future breast cancer[2]. The American Society of Clinical Oncology guideline states that pharmacologic risk reduction with the use of a selective estrogen receptor modulator or an aromatase inhibitor should be discussed with women with a 5-year projected absolute risk of breast cancer of 1.7% or higher[3]. The NCCN guideline for risk reduction recommends consideration of risk-reduction interventions, including the use of pharmacologic agents in women with a 5-year risk of 1.7% or higher and a life expectancy of 10 years or longer [4]. The majority of women with atypical hyperplasia meet this risk criterion with their cumulative risk of approximately 1% per year.

**Method:** We retrospectively reviewed excisional biopsy pathology reports between January 2016 and June 2016 with the diagnosis of atypical ductal or lobular hyperplasia to identify patients with pure atypical hyperplasia. Medical records of these patients were then reviewed to identify the percentage of patients referred to a medical oncologist for chemoprevention discussion and the percentage of patients who received chemoprevention following excisional biopsy.

**Results:** Two hundred seventy six patients with the diagnosis of atypical ductal or lobular hyperplasia were identified. Two hundred and sixteen patients were excluded from the analysis due to the presence of other histologies such as carcinoma in situ and invasive carcinoma. Medical records of the remaining sixty patients with pure atypical hyperplasia were reviewed. Eighteen patients’ charts were unavailable for review. All of the remaining forty two patients had a 5-year breast cancer risk of 1.7% or higher. Five of these patients (8.3%) were referred to a medical oncologist for chemoprevention discussion. Two of these five patients (2.3%) received chemoprevention with tamoxifen. For patients who were not referred to medical oncologist, there was one documented discussion of chemoprevention with patient by her surgical oncologist. One patient underwent prophylactic bilateral mastectomies, and therefore, chemoprevention was not recommended.

**Conclusion:** Multidisciplinary strategies need to be implemented to bridge the gap between guidelines and clinical practices which may lead to improved patient outcomes.

**References:**


**Title:** NEOS: A randomized, open label, phase 3 trial of adjuvant chemotherapy for postmenopausal breast cancer patients who responded to neoadjuvant letrozole: First report of long-term outcome and prognostic value of response to neoadjuvant endocrine therapy

Hiroji Iwata¹, Norikazu Masuda², Tomomi Fujisawa³, Tatsuya Toyama⁴, Shoichiro Ohtani⁵, Yutaka Yamamoto⁶, Masahiro Kashiwaba⁷, Naruto Taira⁸, Takehiko Sakai⁹, Yoshiie Hasegawa¹⁰, Rikiya Nakamura¹¹, Hiromitsu Akabane¹², Yukiko Shibahara¹³, Hiroshi Sasano¹³, Takahiro Yamaguchi¹³ and Yasuo Ohashi¹⁴. ¹Aichi Cancer Center Hospital, Nagoya, Japan; ²NHO Osaka National Hospital, Osaka, Japan; ³Gunma Prefectural Cancer Center, Maebashi, Japan; ⁴Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan; ⁵Hiroshima City Hiroshima Citizens Hospital, Hiroshima, Japan; ⁶Kumamoto University, Kumamoto, Japan; ⁷Breastopia Miyazaki Hospital, Miyazaki, Japan; ⁸Okayama University Hospital, Okayama, Japan; ⁹Cancer Institute Hospital of Japanese Foundation for Cancer Research, Tokyo, Japan; ¹⁰Hirokaki Municipal Hospital, Hirokaki, Japan; ¹¹Chiba Cancer Center, Chiba, Japan; ¹²Hokkaido P.W.F.A.C. Asahikawa-Kosei General Hospital, Asahikawa, Japan; ¹³Tohoku University Graduate School of Medicine, Sendai, Japan and ¹⁴Chuo University, Tokyo, Japan.

**Body:**

**Background:** Whether adjuvant chemotherapy is required for patients (pts) with intermediate-risk endocrine-responsive postmenopausal breast cancer (BC) remains unknown. Sufficient data have not been available about the long-term prognosis of patients with neoadjuvant endocrine therapy (ET). NEOS is a randomized phase III study that assessed the long-term prognosis of estrogen receptor positive (ER+) primary breast cancer (PBC) pts who received neoadjuvant ET with/without adjuvant chemotherapy.

**Methods:** Postmenopausal BC pts with ER+/-HER2 negative, T1c-2, clinically node negative, under 76 years old were enrolled at primary registration. Pts were treated by letrozole (LET) in weeks 24-28 after primary enrollment. Pts experienced progression (PD) during neoadjuvant phase were excluded at randomization and received any systemic therapy driven by investigators before or after surgery. The long-term prognosis was followed in all registered pts including PD pts. Response to neoadjuvant ET was evaluated as complete response (CR), partial response (PR) or stable disease (SD) using calipers, ultrasound and MRI (or CT) at the baseline and end of treatment before surgery. Pts who met eligibility criteria were randomized 1:1 to LET for 4.5-5 years after chemotherapy or LET alone for 4.5-5 years without chemotherapy after surgery. Pts excluded at second registration were treated any systemic therapies driven by investigators. The primary endpoint was disease-free survival (DFS) and secondary endpoints included overall survival (OS), clinical response rate in neoadjuvant phase, pathological response, and breast-conserving surgery rate. The randomization code have been blinded to the investigators.

**Results:** Between May 2008 and June 2013, 904 patients were enrolled at primary registration from 100 institutions in Japan (median follow-up: 4.0 years) and 24 pts were withdrawn during neoadjuvant phase. The median age was 63 years, T1c:37%, T2:63%, and PgR+:78%. Clinical response rates (CR, PR, SD and PD) were 2% (16pts), 48% (421pts), 45% (400pts) and 5% (43pts), respectively and, in each response category, 0% (0/16), 5.5% (23/421), 7.8% (31/400), and 20.9% (9/43) experienced DFS events. DFS in PD pts to neoadjuvant ET were statistically significantly worse than CR, PR, SD and PT (p<0.0001, hazard ratio 4.7 (95% CI:2.3-9.5). The prognosis after surgery in 669 randomized pts was good regardless with/without chemotherapy, forty four pts (6.6%) experienced DFS events after surgery. The predictive markers of PD for neoadjuvant ET were yet unclear among evaluated clinical factors.

**Conclusion:** This is the first report of DFS in the largest neoadjuvant ET trial (NEOS). The DFS of postmenopausal, ER+/HER2-, PBC pts excluding PD pts to neoadjuvant ET is highly good regardless with/without chemotherapy. Neoadjuvant ET with utilization of PD response as a prognostic marker can be considered as a standard treatment option for these patients. Clinical trial information: UMIN000001090.
2017 San Antonio Breast Cancer Symposium

Publication Number: P3-13-04

Title: Change in oncotype DX variables and Ki67 response and outcome in women with ER positive early breast cancer treated with neo-adjuvant anastrozole and fulvestrant

Qamar J Khan¹, Carol J Fabian¹, Bruce F Kimler¹, Anne P O'Dea¹, Joshua Mamen¹, Amanda Amin¹, Jamie Wagner¹, Michelle Springer¹, Stella Baccaray¹ and Priyanka Sharma¹. ¹The University of Kansas Medical Center, Kansas City, KS.

Body: Neo-adjuvant endocrine therapy (NAET) provides opportunity for on-treatment assessment of endocrine responsiveness and need for adjuvant chemotherapy (C) in ER+/HER2 negative breast cancer (BC). We investigated Ki67-IHC response to NAET, the 21-gene Recurrence Score (RS; Oncotype DX), and Postoperative Endocrine Prognostic Index (PEPI) as predictors of response and recurrence in a neoadjuvant trial of fulvestrant (F) + anastrozole (A). The combination had been shown to improve survival compared to A alone in ER+ metastatic breast cancer.

Design: Single arm phase II trial of postmenopausal women with clinical stage II/III ER+/HER2 negative BC and RS ≤25. Following initial biopsy (CNB) women received NAET for 4 months: A 1mg (PO) daily continuously from day 1 until surgery + F 500mg IM on day 1, 14 and 28 of cycle 1, and on the last day of 3 subsequent 28 day cycles (total 6 doses of F) followed by surgery. Ki67-IHC and RS (weighted heavily by gene expression of ESR1, HER-2, Ki67, and PR) were assessed at baseline and at surgical resection (3-4 weeks after last F). Chemotherapy was allowed at physician discretion and recommended for PEPI>0. All patients received adjuvant A.

Results: 51 patients were screened and RS was performed on initial CNB with a success rate of 96%. 7 were not eligible due to RS>25. 42 patients were enrolled. 18 (49%) had PEPI score of 0 at surgery. Median RS was 12 at baseline and 17 at surgery for the 36 patients with evaluable tissue at both time points. 28/36 (78%) patients had numeric increase in RS at surgery and 5/36 had RS > 25 at surgery. Increase in RS is likely driven by decrease in ESR1 gene expression (p=0.063). Median RS at surgery but not at baseline was associated with PEPI score of 0 (p=0.02). Ki67 gene expression at surgery was lower in patients with PEPI 0 (p=.003) whereas ESR1 expression was higher (p=.024). Median Ki67-IHC was 5% (mean 7.6%) at baseline and 1% (mean 2.8%) at surgery for the 37 patients with evaluable tissue at both time points. 35 of these 37 (94%) had decrease in Ki67-IHC from baseline to surgery; only 2/37 had an increase in Ki67-IHC at surgery. 22% of women received adjuvant chemotherapy. At median follow up of 61 months, only 3/42 patients have had a recurrence: the two patients with an increase in Ki67-IHC from baseline to surgery had a recurrence; plus a third who was taken off trial during therapy due to clinical progression and received C prior to surgery.

Conclusions: 4 months of neo-adjuvant anastrozole + fulvestrant results in decrease in Ki67-IHC in 94% of women with stage II/III ER+ HER2 negative breast cancer. No patients with decrease in Ki67-IHC at surgery have had a breast cancer recurrence. The majority of patients treated with this regimen do not need chemotherapy. A small number of patients with on-therapy increase in Ki67-IHC are at high risk of recurrence. Oncotype DX RS and expression of some of the component genes (ESR1 and Ki67) change with NAET. Expression of ESR1 and Ki67 genes at surgery after NAET is associated with PEPI score of 0 and should be explored as potential response biomarkers in NAET trials.
Title: Long-term outcome of neoadjuvant endocrine therapy followed by breast conserving surgery

JD Yau\textsuperscript{1}, Arran K Turnbull\textsuperscript{1}, Lorna Renshaw\textsuperscript{2}, Jane Keys\textsuperscript{2}, Alexander Leeper\textsuperscript{2}, Jeremy S Thomas\textsuperscript{3} and J Michael Dixon\textsuperscript{2}. \textsuperscript{1}Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, United Kingdom; \textsuperscript{2}Edinburgh Breast Unit, Western General Hospital, Edinburgh, United Kingdom and \textsuperscript{3}Western General Hospital, Edinburgh, United Kingdom.

Body: Background: Neoadjuvant therapy (NET) in women with large or locally advanced estrogen receptor (ER)-rich breast cancers (BC) allows the option of breast-conserving surgery (BCS) + radiotherapy (RT). The aim was to study the long term safety of this strategy.

Methods: 280 postmenopausal women (median age 77, range 50-95, table 1) with ER-rich BC had BCS after NET (median duration 4.8 mths, range 1.7-42.8 mths). 221 (79%) received only letrozole. 59 (21%) began on letrozole and were switched to anastrozole, exemestane or tamoxifen due to adverse events or lack of clinical response. 200 patients (71%) had adjuvant RT (RTgroup) and 25 (9%), adjuvant chemotherapy. Median follow-up = 5.5 years.

Table 1: Clinical characteristics of patients.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour Size</td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>31 (11%)</td>
</tr>
<tr>
<td>T2</td>
<td>169 (60%)</td>
</tr>
<tr>
<td>T3</td>
<td>14 (5%)</td>
</tr>
<tr>
<td>T4</td>
<td>56 (20%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>10 (4%)</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>36 (13%)</td>
</tr>
<tr>
<td>2</td>
<td>153 (55%)</td>
</tr>
<tr>
<td>3</td>
<td>70 (25%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>21 (7%)</td>
</tr>
<tr>
<td>Nodes</td>
<td></td>
</tr>
<tr>
<td>+ve</td>
<td>101 (36%)</td>
</tr>
<tr>
<td>-ve</td>
<td>177 (63%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>3 (1%)</td>
</tr>
<tr>
<td>ER Allred</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>15 (5%)</td>
</tr>
<tr>
<td>7</td>
<td>62 (22%)</td>
</tr>
<tr>
<td>8</td>
<td>203 (73%)</td>
</tr>
</tbody>
</table>

Results:
254 patients had NET response data. 74% had a clinical response. NET response was higher for grade 1 (84%) than for 2 (73%) or 3 (72%) cancers. Actuarial local recurrence rates (LRR) were 8% (95%CI±0.04) and 12% (95%CI±0.06) at 5 & 10 years. Actuarial overall BC...
Recurrence rates were 14% (95% CI ± 0.04) and 27% (95% CI ± 0.12) at 5 & 10 years, with BCS death rates of 7% (95% CI ± 0.04) and 14% (95% CI ± 0.10) at 5 & 10 years, showing only half with recurrence died from BC. Crude all-cause mortality but not BC-specific survival (BCSS) favoured those who had adjuvant RT (P < 0.001) or chemotherapy (P = 0.006). The 15-year rate was 50.9%, while BCS death rate was only 7.3%.

Positive nodes were associated with worse overall recurrence free survival (RFS) (P = 0.007) but not local RFS or BCSS. Tumour size was not associated with RFS or BCSS. Tumour grade was not associated with RFS but grade 3 patients had a lower BCSS (P = 0.002) compared to patients grade 1/2 cancers. RT was associated with improved LRR (P < 0.0001) and overall RR (P = 0.038): 5 & 10 year in RT group were 5% (95% CI ± 0.04) + 7% (95% CI ± 0.04) vs 9% (95% CI ± 0.12) + 31% (95% CI ± 0.24) in no-RT group. The 5 & 10 year ORR in the RT group was 14% (95% CI ± 0.06) and 39% (95% CI ± 0.16) vs 28% (95% CI ± 0.16) + 38% (95% CI ± 0.24) in the no-RT group. Although differences were not significant, BCSS was higher in the no-RT group: 5 & 10 yearly BCSS rates were 10% (95% CI ± 0.04) and 20% (95% CI ± 0.12) vs 6% (95% CI ± 0.08) + 12% (95% CI ± 0.14) in RT group.

16/67 patients with T3/4 cancers with no RT had lower overall RFS (P = 0.018) but no difference in local RFS. 13/98 patients with node +ve disease with no RT had lower LRR (P = 0.002) and overall RR (P = 0.024). 54/169 node -ve patients with no RT had lower LRR (P = 0.019) but similar overall RR. 50/183 patients with grade 1/2 cancers had no RT and had lower LRR (P < 0.0001) and overall RR (P = 0.049). BCSS was not associated with RT use in subgroups related to tumour size, node status or grade.

Discussion:
• Response to NET is not worse in ER rich grade 3 or node positive cancers.
• After NET, BCS and RT provides excellent LRR.
• BCSS rates were low; most died of other causes.
• NET followed by BCS and RT is safe even for grade 3 and node positive cancers.
• BCS alone provides adequate disease control for majority with significant co-morbidities.
2017 San Antonio Breast Cancer Symposium

Publication Number: P3-13-06

Title: Tailored neoadjuvant endocrine and chemo-endocrine therapy for postmenopausal patients with estrogen receptor-positive human epidermal growth factor receptor 2-negative primary breast cancer

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Body: Aims We investigated the efficacy and safety of initial neoadjuvant endocrine therapy with exemestane (EXE) alone followed by subsequent tailored treatment with EXE alone for responders or EXE plus oral metronomic cyclophosphamide (CPA) for non-responders.

Methods In this multicenter open-label phase II study, we enrolled postmenopausal patients with primary invasive estrogen receptor (ER)-positive, HER2-negative, stage I–IIIA (T1c–T3 N0–2 M0) breast cancer and Ki67 index ≤ 30%. Patients first received EXE 25mg/day for 12 weeks. Based on clinical response and change in Ki67 index in response to the initial therapy, patients who achieved complete response (CR), partial response (PR) with Ki67 index ≤5% after treatment, or stable disease (SD) with Ki67 index ≤5% both before and after treatment were defined as responders. Non-responders were defined as patients with PR and Ki67 index >5% after treatment, or SD and Ki67 index >5% before or after treatment. For the subsequent 24 weeks, responders continued the EXE monotherapy (continued EXE group), whereas non-responders switched to combination therapy with EXE plus CPA 50mg/day (EXE+CPA group). The primary endpoint was clinical response (CR and PR) at weeks 24 and 36.

Results A total of 59 patients (median age 69 years, range 53–86 years) were enrolled between January 2011 and July 2015. After exclusion of 3 (2 with progressive disease, 1 with an adverse event, AE) who discontinued treatment in the initial 12-week EXE monotherapy period, 56 remained enrolled to receive subsequent treatment. After 8–12 weeks of the initial EXE monotherapy, 14 patients were classified as responders (9 with PR and Ki67 index ≤5% after treatment; 5 with SD and Ki67 index ≤5% before and after treatment), whereas 42 were classified as non-responders (3 with PR and Ki67 index >5% after treatment; 39 with SD and Ki67 index >5% before or after treatment). Clinical response rates at weeks 24 and 36 were 85% (12/14, 95%CI 57.2–98.2%) and 76% (10/13, 95%CI 46.2–95.0%), respectively, in the continued EXE group, and 56% (23/41, 95%CI 39.7–71.5%) and 76% (30/39, 95%CI 60.7–88.9%), respectively, in the EXE+CPA group. At week 36, no significant difference was found in median Ki67 index between the continued EXE and EXE+CPA groups (3.5% and 4.0%, respectively). The proportion of patients with preoperative endocrine prognostic index (PEPI) 0 was also similar between the continued EXE and EXE+CPA groups (21.4% and 23.8%, respectively). The breast-conserving surgery rate was 71.4% and 69.0%, respectively. Grade 3 AEs were elevated liver enzymes (1 patient) in the continued EXE group, and gastritis, hypertriglyceridemia, and bone mineral density loss (1 patient each) in the EXE+CPA group.

Conclusion Switching from EXE monotherapy to EXE+CPA combination therapy based on clinical response and biological response (change in Ki67 index) to initial therapy improved subsequent clinical response in non-responders. Favorable clinical response to EXE alone was maintained in responders. Tailored neoadjuvant endocrine and chemo-endocrine therapy was shown to be effective in postmenopausal ER-positive breast cancer patients. (JBCRG-11CPA; UMIN000004751)
Title: Effects of adding budesonide or colestipol to loperamide prophylaxis on neratinib-associated diarrhea in patients with HER2+ early-stage breast cancer: The CONTROL trial

Sara Hurvitz¹, Arlene Chan², Nicholas Iannotti³, Emad Ibrahim⁴, Jo Chien⁵, Nancy Chan⁶, Andrew Kellum⁷, Vincent Hansen⁸, Gavin Marx⁹, S DiSean Kendall¹⁰, Mary Wilkinson¹¹, Aurelio Castrellon¹², Rolando Ruiz¹³, Pearl Fang¹³, Daniel Hunt¹³, Susan Moran¹³, Elizabeth Olek¹³, Carlos H Barcenas¹⁴ and Hope S Rugo⁵.¹

¹UCLA Hematology / Oncology Clinical Research Unit, Los Angeles, CA; ²Breast Cancer Research Centre-Western Australia and Curtin University; ³Hematology Oncology Associates of the Treasure Coast; ⁴Center for Surgical and Specialty Care, Redlands; ⁵UCSF Helen Diller Family Comprehensive Cancer Center; ⁶Rutgers Cancer Institute of New Jersey; ⁷North Mississippi Medical Center Hematology and Oncology Services; ⁸Northern Utah Associates; ⁹Sydney Adventist Hospital; ¹⁰Utah Cancer Specialists Research; ¹¹Inova Medical Group; ¹²Memorial Healthcare System; ¹³Puma Biotechnology Inc and ¹⁴MD Anderson Cancer Center.

Body: Background: Neratinib is an irreversible pan-HER tyrosine kinase inhibitor. Results from the randomized, placebo-controlled, Phase III ExteNET study demonstrated that neratinib significantly improves 24-month iDFS in patients (pts) with trastuzumab-treated early-stage HER2+ breast cancer (HR 0.67; 95% CI 0.50–0.91; p=0.009) [Chan et al. Lancet Oncol 2016]. In ExteNET, loperamide prophylaxis was not mandated, and diarrhea was the most commonly observed toxicity (grade 3, 39.8%). To reduce neratinib-associated diarrhea, high-dose loperamide prophylaxis given with the first 1–2 cycles of neratinib has been incorporated into all neratinib clinical trials; available data suggest that loperamide prophylaxis reduces the incidence and median cumulative duration of higher-grade neratinib-related diarrhea. CONTROL is an international, open-label, sequential cohort, phase II study investigating the effects of prophylaxis with loperamide ± the long-acting corticosteroid budesonide or bile acid sequestrant colestipol on neratinib-associated diarrhea. We present updated data from this study.

Methods: Pts with stage 1–3c HER2+ breast cancer who completed trastuzumab-based adjuvant therapy within 1 year were eligible. All pts received oral neratinib 240 mg once daily for 1 year + oral loperamide prophylaxis for 1 or 2 cycles (1 cycle = 28 days) ± budesonide or colestipol for the first cycle (see table). Adverse events were graded according to NCI-CTCAE (v4.0). Primary endpoint: incidence of grade ≥3 diarrhea.

Results:

<table>
<thead>
<tr>
<th>Study</th>
<th>CONTROL</th>
<th>ExteNET³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cohort or study arm</td>
<td>Loperamide</td>
<td>Budesonide</td>
</tr>
<tr>
<td>Antidiarrheal prophylaxis</td>
<td>Loperamide¹,²</td>
<td>Budesonide + loperamide²,³</td>
</tr>
<tr>
<td>N (at data cut-off)</td>
<td>137⁶</td>
<td>64</td>
</tr>
<tr>
<td>Diarrhea, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any grade</td>
<td>78.1</td>
<td>79.7</td>
</tr>
<tr>
<td>Grade 1</td>
<td>24.1</td>
<td>25.0</td>
</tr>
<tr>
<td>Grade 2</td>
<td>23.4</td>
<td>29.7</td>
</tr>
<tr>
<td>Grade 3</td>
<td>30.7</td>
<td>25.0</td>
</tr>
<tr>
<td>Grade 4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Median cumulative duration of diarrhea, days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any grade</td>
<td>12.0</td>
<td>13.0</td>
</tr>
<tr>
<td>Grade ≥2</td>
<td>4.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Grade ≥3⁷</td>
<td>3.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Median episodes of diarrhea per patient, n</td>
<td>(\text{Any grade})</td>
<td>(\text{Grade } \geq 2)</td>
</tr>
<tr>
<td>-----------------------------------------</td>
<td>----------------</td>
<td>----------------</td>
</tr>
<tr>
<td>(\text{Any grade})</td>
<td>2.0</td>
<td>4.0</td>
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<tr>
<td>(\text{Grade } \geq 2)</td>
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<td>2.0</td>
</tr>
<tr>
<td>(\text{Grade } \geq 3)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Neratinib discontinuation (due to diarrhea), %</td>
<td>20.4</td>
<td>9.4</td>
</tr>
<tr>
<td>Hospitalization, %</td>
<td>1.5</td>
<td>0</td>
</tr>
<tr>
<td>Median duration of neratinib treatment, mo</td>
<td>11.0</td>
<td>6.3</td>
</tr>
</tbody>
</table>

1. Oral loperamide 4 mg, then 2 mg q4h d1-3, then 2 mg q6-8h d4-56 (original); 2. Oral loperamide 4 mg, then 4 mg tid d1-14, then 4 mg bid d15-56 (modified); 3. Oral budesonide 9 mg qd d1-28; 4. Oral colestipol 2 g qd d1-28; 5. Chan et al. *Lancet Oncol* 2016; 6. Original,\(^1\) n=28; modified,\(^2\) n=109; 7. Grade 4 events: CONTROL, n=0; ExteNET, n=1; 8. Treatment ongoing in all CONTROL cohorts. CONTROL data cut-off: Apr 2017.

**Conclusions:** A structured loperamide prophylactic regimen for 1 or 2 cycles reduces the incidence, severity and duration of neratinib-associated diarrhea compared with events observed in the ExteNET trial. Adding budesonide or colestipol appears to further diminish the duration and number of episodes of diarrhea and improves neratinib tolerability. Final analysis of the CONTROL study will be performed when all pts have completed 12 months of neratinib therapy. Updated data will be available at the meeting.

Clinicaltrials.gov: NCT02400476.
**Title:** Incidence of venous thromboembolism in patients with hormone receptor-positive HER2-negative metastatic breast cancer treated with CDK 4/6 inhibitors: A systematic review and meta-analysis of randomized controlled trials

Kyaw Z Thein¹, Myo H Zaw², Aung M Tun², Catherine Jones¹, Saba Radhi¹, Fred Hardwicke¹ and Thein H Oo². ¹Texas Tech University Health Sciences Center, Lubbock, TX; ²The Brooklyn Hospital Center, New York, NY and ³The University of Texas MD Anderson Cancer Center, Houston, TX.

**Body: Background:**

The cyclin dependent kinases (CDK) along with their partners, the cyclins, have a crucial role in regulation of the cell cycle. Several CDK-targeted agents have been employed in hormone receptor positive metastatic breast cancer (MBC) with noteworthy safety concerns. Nevertheless, the impact of this agent on risk of venous thromboembolism (VTE) remains uncertain. We performed a systematic review and meta-analysis of randomized controlled trials (RCT) to determine the risk of VTE among patients with hormone receptor-positive HER2-negative MBC treated with CDK 4/6 inhibitors.

**Methods:**

We systematically conducted a comprehensive literature search using MEDLINE, EMBASE databases and meeting abstracts through June 2017. Trials that mention deep vein thrombosis and pulmonary embolism as adverse effects were incorporated in the analysis. The primary meta-analytic approach was a fixed effects model using the Mantel-Haenszel (MH) method. It was used to calculate the estimated pooled risk ratio (RR), and risk difference (RD) with 95% confidence interval (CI). Pooled VTE rates were estimated as follows: we multiplied the median follow-up duration by the sample size. Crude study-specific VTE rates were then calculated by dividing the number of incident VTE cases by the total number of person-months follow-up.

**Results:**

A total of 2671 patients with hormone receptor-positive HER2-negative MBC from four phase 3 studies and one phase 2 study were eligible for analysis. The study arm used palbociclib-letrozole, palbociclib-fulvestrant, ribociclib-letrozole and abemaciclib-fulvestrant while the control arm utilized placebo in combination with letrozole or fulvestrant. The $I^2$ statistic for heterogeneity was 13.6, and the heterogeneity $X^2$ (Cochran’s Q) was 4.6 ($P=0.3$), suggesting homogeneity of results among the randomized trials. The VTE incidence was 24 (1.46%) in CDK 4/6 group vs 4 (0.39%) in control group. The pooled RR for VTE was 2.736 (95% CI: 1.115 – 6.714, $P = 0.028$) and the absolute RD was 0.010 (95% CI: 0.002 – 0.018, $P = 0.010$) according to the fixed effects model. By the random effects model, the pooled RR was 2.411 (95% CI: 0.809 – 7.181, $P = 0.114$) and RD was 0.009 (95% CI: 0.0 – 0.019, $P = 0.048$). Over median follow up of 36 months, the RR for VTE was 3.792 (95% CI: 1.838 – 7.822, $P < 0.0001$) and RD was 0.024 (95% CI: 0.014 – 0.034, $P < 0.0001$) with the fixed effects model. By the random effects model, the pooled RR for VTE was 4.248 (95% CI: 0.952- 18.959, $P = 0.058$) and RD was 0.026 (95% CI: 0.004 – 0.021, $P < 0.0001$). The pooled rate of VTE among CDK 4/6 group was 2.99 per person years compared to 0.50 per person years among control arm.

**Conclusion:**

Approximately 1% of patients on letrozole or fulvestrant alone developed VTE in previous studies. Our meta-analysis demonstrated that the addition of CDK 4/6 inhibitors to letrozole or fulvestrant, contribute to higher incidence of VTE. More randomized trials are required to determine the actual relation and definitive incidence of VTE, a major cause of morbidity and mortality among these patients.
2017 San Antonio Breast Cancer Symposium

Publication Number: P3-14-03

Title: PRESAGE : Prospective multicenter feasibility study of fertility preservation before neoadjuvant or adjuvant chemotherapy for breast cancer: Preliminary results

Virginie Bordes¹, Camille Frick¹, Florence Leperlier², Aliette Dezellus¹, Pascaline De Blay³, Fabienne Maigne³, Baptiste Sauterey⁴, Paule Augereau⁴, Claudia Lefeuvre-Plesse⁵, Mario Campone¹ and Sophie Mirallie². ¹ICO René Gauducheau, Nantes, France; ²CHU, Nantes, France; ³CHD Vendée, La Roche Sur Yon, France; ⁴ICO Paul Papin, Angers, France and ⁵Centre Eugene Marquis, Rennes, France.

Body: Background: Breast cancer is the most frequent form of cancer for young women. For these patients, breast cancer is generally more aggressive and chemotherapy is more often needed. Chemotherapy is commonly associated with amenorrhea and a decrease of ovarian reserve depending on the patient's age, agents and dose. There are no guidelines to prevent subfertility for young women on cytotoxic treatments. Embryo, oocyte and ovarian tissue cryopreservation are the three options to preserve fertility. Embryo and oocyte cryopreservation require controlled ovarian stimulation (COS). The use of COS is associated with an increase of estradiol levels. It led to develop protocols using Tamoxifene or Letrozole combined with FSH to protect patients of the potential deleterious effects of the COS. We are currently conducting a study: PRESAGE, the first French prospective multicenter feasibility study of fertility preservation by COS combined with Tamoxifene and oocyte +/- embryo cryopreservation before neoadjuvant (NAC) or adjuvant (AC) chemotherapy for breast cancer.

Material and method: Prospective multicenter study for patients of less than 40 years, with a breast cancer for whom a treatment of NAC or AC is indicated and who wish to preserve their fertility. The main objective: To evaluate the feasibility of a COS associating Tamoxifène with FSH followed by an oocyte more and less embryo cryopreservation. The secondary objectives: evaluation of the average deadline prior to the beginning of the chemotherapy, the impact of the type of COS (depending on the phase of the menstrual cycle, conventional-start or random-start COS protocol) on the number and the quality of oocytes harvested. Statistical analysis was performed using BiostaTGV software.

Results: The 50 first patients were included between February 2014 and May 2016. Mean age of the patients was 31 years, half of it was nulliparous (25/50) and 20 % (10/50) were single. They presented mainly SBR II (23/50, 46 %) or III (25/50, 50 %) lesions, ER + (34/50, 68 %). 13 patients benefited from a NAC and 37 of an AC. In the NAC group, the time between the first oncologist's consultation and the fertility specialist's consultation was 3,5 +/- 3,9 days and the time between the first oncologist's consultation and the beginning of the chemotherapy was 24,5 +/- 6 days. The success rate of the COS procedure was 88 % (44/50) with no significant difference between the groups according to the type of COS (conventional-start vs. random-start p = 0.61) but there was a significant difference according to the type of chemotherapy (AC vs. NAC p= 0.03). In the 44 patients who had oocyte retrieval, the number of oocytes was 11,6 +/- 6,3 and an IVF was performed with 11,3% of patients (5/44) with 5,4 +/- 4,6 embryos obtained. We have found no impact of the type of chemotherapy or the type of COS on the number of oocytes or embryos preserved.

Conclusion: with an 88% success rate, our study suggests that COS with Tamoxifene and FSH is feasible before adjuvant or neoadjuvant chemotherapy with less success in this context but without any impact on the number of oocytes collected. We hope that these preliminary results will be confirmed at the end of the study.
2017 San Antonio Breast Cancer Symposium

Publication Number: P3-14-04

Title: Hepatic pseudocirrhosis in breast cancer – analyses of clinical and prognostic factors

Dharmesh Gopalakrishnan1 and Jame Abraham2. 1Cleveland Clinic Foundation, Cleveland, OH and 2Taussig Cancer Institute, Cleveland Clinic Foundation, Cleveland, OH.

Body: Background
Hepatic pseudocirrhosis (HP) is a rare entity characterized by radiological changes in the liver resembling cirrhosis (lobular contour, nodularity, capsular retraction, and atrophy), reported most frequently in patients (pts) with treated metastatic breast cancer (BC). Cases in literature have been associated with poorer prognosis than true cirrhosis with fatal outcome nearly always in a few months. Little is known about factors which predispose to HP or influence its prognosis.

Methods
In this retrospective observational study, we reviewed abdominal radiology reports (CT, MRI, and ultrasound scans) of all our pts with BC diagnosed between 01/01/07 and 12/31/16, to identify those with HP. Cirrhosis on baseline imaging, pre-existing chronic liver disease, hepatitis B/C, and heavy alcohol use were exclusion criteria. Routine descriptive statistical measures were employed. Multiple regression was used to analyze factors affecting survival. p <0.05 was considered significant.

Results
We identified 86 pts with HP who satisfied inclusion/exclusion criteria. All were females, 89.5% were Caucasian. 83.7% of primary tumors were ER+, 58.1% were PR+ and 17.4% were HER2+. 25.6% had an invasive lobular component. The vast majority had distant metastases (mets) (97.7%) and liver mets (94.2%) at the time of diagnosis of HP, though 52.3% had stage I/II BC on initial presentation. Median size of the largest liver met was 30.0 mm and 87.2% pts had >10 liver mets. Most pts were heavily pre-treated before the manifestation of HP – 84.9% had received hormonal therapy (median cumulative duration of 35 months each of tamoxifen and aromatase inhibitors), 18.6% got anti-HER2 therapy (median duration of 15 months), and 96.5% received chemotherapy (81.4% had received ≥2 lines of chemotherapy). Median interval from diagnosis of BC to detection of HP was 6.3 years (IQR 2.9-9.6). 36% had ≥1 signs of portal hypertension (splenomegaly 15.1%, esophageal/gastric varices 18.6%, variceal bleeding 9.3% and ascites with high albumin gradient 34.9%), while 48.8% had ≥1 signs of hepatocellular failure (jaundice 47.7%, coagulopathy 30.2%, hepatic encephalopathy 14.0%). Detection of HP often led to changes in BC therapy – chemotherapy was changed in 46.5% pts, stopped in 24.4%, hormonal therapy was changed in 18.6%, stopped in 15.1%, and hospice care was initiated in 19.8% pts. Status of BC and liver mets at initial detection of HP were variable – 53.5% had disease progression while 45.3% had continued response; 40.7% had enlarging, 23.4% had stable and 30.9% had shrinking liver mets. Median survival after detection was HP was 3.6 months (1.1-11.5). Death was due to BC-progression in 83.4% pts and HP-related complications (GI bleeding, hepatic failure) contributed in 28.6% pts. Using multiple regression, lower albumin level and a higher number of metastatic sites at initial detection of HP were found to be independently associated with shorter post-HP survival (R² 16.6%, F(2,74) 7.37, p=0.001).

Conclusions
We report the largest series, till date, of BC patients with HP, an entity which can be complicated by portal hypertension and hepatocellular failure, and with marked impact on BC survival and management. Studies are needed to identify risk factors and management strategies.
Title: Safety of granulocyte colony-stimulating factors and their biosimilars: A meta-analysis of randomized clinical trials in breast cancer patients receiving cytotoxic chemotherapy

Andriy Krendyuk1, Edoardo Botter2,3 and Giuseppe Curigliano4. 1Hexal AG, Holzkirchen, Germany; 2Women and Children's Division, Norwegian National Advisory Unit on Women's Health, Oslo University Hospital, Oslo, Norway; 3Cancer Registry of Norway, Oslo, Norway and 4European Institute of Oncology, Division of Experimental Therapeutics, Oslo, Norway.

Body: Background: Neutropenia is a common adverse event reported in cancer patients undergoing cytotoxic chemotherapy. Granulocyte colony-stimulating factors (G-CSFs) such as filgrastim and pegfilgrastim are widely used to prevent neutropenia. Several biosimilars of G-CSF are now available. Biosimilar development involves a series of comparisons between the proposed biosimilar and reference performed in a step-wise fashion to eliminate any concerns regarding the similarity of the medicines. Randomized clinical trials (RCTs) are then performed to confirm that the reference product and its biosimilar provide the same clinical efficacy and safety. Patients with breast cancer (BC) are the most sensitive population in which to confirm similarity of G-CSF biosimilars, however there are some differences between clinical studies. The aim of this meta-analysis was to compare the safety profiles of approved or proposed G-CSF biosimilars (filgrastim or pegfilgrastim) with reference G-CSF in patients with BC.

Methods: A Medline literature search up to March 2017 identified randomized clinical trials (RCTs) comparing biosimilar G-CSF to reference in BC patients. Safety analyses included calculation of risk ratios for bone pain events, myalgia events and serious adverse events. Random effect models were fitted to obtain the pooled estimates of the risk ratio for the outcomes and their corresponding 95% confidence intervals (CIs).

Results: Eight eligible RCTs were included in this meta-analysis. Risk ratios for bone pain events (risk ratio 1.01 [95% CI -0.76, 1.34]; Table), myalgia events (risk ratio 0.94 [95% CI 0.63, 1.40]) and serious adverse events (risk ratio 1.01 [95% CI 0.76, 1.34]) showed no significant differences between reference and biosimilar G-CSF.

Risk ratios for bone pain events

<table>
<thead>
<tr>
<th>Study and year of publication</th>
<th>Reference G-CSF / Biosimilar G-CSF</th>
<th>Events</th>
<th>Total</th>
<th>Weight</th>
<th>IV, Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blackwell 2015</td>
<td>Filgrastim / Filgrastim</td>
<td>33</td>
<td>107</td>
<td>29.9%</td>
<td>0.87 [0.59, 1.27]</td>
</tr>
<tr>
<td>Blackwell 2016</td>
<td>Pegfilgrastim / Pegfilgrastim</td>
<td>10</td>
<td>155</td>
<td>11.8%</td>
<td>0.58 [0.27, 1.23]</td>
</tr>
<tr>
<td>Harbeck 2016</td>
<td>Pegfilgrastim / Pegfilgrastim</td>
<td>7</td>
<td>159</td>
<td>7.3%</td>
<td>0.86 [0.32, 2.33]</td>
</tr>
<tr>
<td>Waller 2010</td>
<td>Filgrastim / Filgrastim</td>
<td>48</td>
<td>183</td>
<td>21.1%</td>
<td>1.56 [0.94, 2.59]</td>
</tr>
<tr>
<td>Waller 2016</td>
<td>Pegfilgrastim / Pegfilgrastim</td>
<td>51</td>
<td>127</td>
<td>29.7%</td>
<td>1.12 [0.76, 1.65]</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td></td>
<td>731</td>
<td>579</td>
<td>100%</td>
<td>1.01 [0.76, 1.34]</td>
</tr>
</tbody>
</table>

Heterogeneity: Chi² = 5.86, df = 4 (P = 0.21); I² = 32% Test for overall effect: Z=0.07 (P=0.94)

Conclusions: This meta-analysis showed no differences in the safety profile of biosimilar and reference G-CSF in breast cancer patients.
The prevention and management of chemotherapy-induced peripheral neuropathy in breast cancer patients: A systematic review

Kate E Roberts¹,², Sophie Feng¹,², India Adsett³, Kirsty Rickett² and Natasha Woodward¹,². ¹Mater Adults Hospital, Brisbane, Queensland, Australia; ²University of Queensland, Brisbane, Queensland, Australia and ³Griffith University, Southport, Queensland, Australia.

Body: Background: Chemotherapy induced peripheral neuropathy (CIPN) remains a common treatment related adverse event for breast cancer (BC) patients. CIPN interferes with the ability to complete chemotherapy & therefore potentially compromises survival outcomes. CIPN can worsen health-related quality of life and also add to the ongoing morbidity experienced by BC survivors. Previously, systematic reviews & ASCO guidelines have examined CIPN across many cancer types & different chemotherapies, which may lead to the introduction of confounding factors not relevant to BC patients. A recent prospective cohort also identified other specific lifestyle factors associated with CIPN in BC patients (1). From our search, a systematic review specifically looking at CIPN in BC patients has not previously been undertaken.

Methods: We conducted a systematic search in PubMed, EMBASE, CINAHL and CENTRAL. Clinical trials and observational studies for all potential pharmacological and non-pharmacological interventions were included. Risk of bias for full-text papers was assessed using the Cochrane Risk of Bias Assessment or the modified Newcastle Ottawa score.

Results: Of 706 records being identified, 601 unique citations were screened with 21 full text papers retrieved for assessment, and 16 studies included in the qualitative assessment. We identified 10 randomised controlled trials (RCTs), one observational cohort study, and five controlled before-and-after studies (CBA). Pharmacological interventions which were investigated included calcium/magnesium infusion, glutamine, amifostine, goshajinkigan, omega 3 fatty acids, acetyl-L-carnitine, pregabalin, alpha-lipoic acid and minocycline. Non-pharmacological interventions included body mass index & lifestyle factors, electroacupuncture, exercise, limb hypothermia, carbon dioxide limb bathing and limb compression therapy. All trials identified were investigating primary prevention of CIPN in the setting of taxane chemotherapy. Improvements in the incidence of CIPN were reported with omega 3 fatty acids, glutamine and alpha-lipoic acid, but only one of these studies is a published RCT. In two of the studies, the benefit was identified only on physical examination, but no significant benefit with nerve conduction (NCS) testing. On subgroup analysis of trials investigating exercise, there was a benefit in the use of high intensity exercise versus low intensity exercise, particularly in patients <50 years old within a healthy weight range. Mixed results were seen with goshajinkigan. An increase in CIPN was seen with antioxidant use, electroacupuncture and acetyl L carnitine. Majority of the RCTs were associated with a high overall risk of bias.

Conclusions: There has been a paucity of research on CIPN in BC patients. There were no interventions identified in this systematic review which show a clear clinical benefit in the prevention of CIPN in BC patients. BC patients may have specific lifestyle and hormonal factors which influence CIPN and this should prompt ongoing specific patient focussed research within this subgroup with large, blinded, randomised controlled trials.

Early detection of chemotherapy-induced cardiotoxicity in breast cancer patients

Carmen Salvador-Coloma¹,³, Amparo Hernández¹, Sandra Tejedor¹, Vicente Miró², Laura Palomar², Antonio Salvador², Pilar Sepúlveda¹ and Ana Santaballa³. ¹Instituto de Investigación Sanitaria La Fe, Valencia, Spain; ²Servicio de Cardiología. Hospital Universitario y Politécnico La Fe, Valencia, Spain and ³Servicio de Oncología Médica. Hospital Universitario y Politécnico La Fe, Valencia, Spain.

**Body: BACKGROUND**

The incidence of cardiotoxicity in patients receiving treatment for breast cancer is unknown. There is not enough evidence about early detection and appropriate management of cardiotoxicity. The aim of this study is to identify early markers of risk of cardiac toxicity.

**MATERIAL AND METHOD**

Prospective study was conducted between 2014 and 2017 based on a cohort of 97 patients diagnosed with breast cancer treated with chemotherapy. Analytical biomarkers (natriuretic peptide, ultra-sensitive T troponin), echocardiogram parameters (left ventricular ejection fraction (LVEF) and global longitudinal strain (GLS)) and electrocardiogram were performed. Analytical biomarkers were measured each chemotherapy cycle and cardiology test were performed before starting chemotherapy, 3 months afterwards, and then every six months during 5 years. Cardiotoxicity was defined as a reduction in basal LVEF >10% with LVEF<55% in asymptomatic patients or >5% with LVEF<55% in symptomatic patients.

**RESULTS**

Patients characteristics are shown in table 1

<table>
<thead>
<tr>
<th>Variables</th>
<th>No cardiotoxicity (n=88)</th>
<th>Cardiotoxicity (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (women/men)</td>
<td>88/0</td>
<td>12/0</td>
</tr>
<tr>
<td>Median age (range)</td>
<td>53 years (29-79)</td>
<td>47 years (37-70)</td>
</tr>
<tr>
<td>Smoker (former smoker)</td>
<td>13 (3)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Arterial hypertension</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>Dyslipemia</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>Diabetes</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Previous chemotherapy</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Prior mediastinal radiation therapy</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

. All patients had the basal LVEF in normal range. Median follow-up was 26.5 months (13.5-39.6 months). A total of 10.3% had cardiotoxicity with reduction in basal LVEF >10% with LVEF<55% being asymptomatic and 2.1% were symptomatic. Five of these patients were treated with heart failure therapy: 5 with ACE (angiotensin converting enzyme) inhibitors and 3 with beta- blockers. Two of those (40%) who received specific treatment recovered basal LVEF-levels, 2 maintained LVEF dysfunction, and 1 died during follow-up due to tumor-related causes. In 83.3% of patients, cardiotoxicity occurred within the first year of follow-up. In 50 patients SLG was calculated, in 30% it was lower than -12% in some measurement phase. In 5 cases the LVEF fell below 55% and the LRP decreased by 12% coincided. The others patients, although they did not develop cardiotoxicity according to the established criteria, a decrease of the LVEF is observed during the treatment and in the first control, between 3-7%, which subsequently tends to recover spontaneously.

miRNA 21-5p, miRNA-133b, miRNA 210-3p, miRNA 423-5p, and miRNA-663b were analyzed. A model has been evaluated where a correlation between the levels of miRNA-133b, miRNA-21-5p and miRNA-210-3p and the decrease of LVEF in relation to
treatment was observed.

CONCLUSIONS
Control by echocardiography and serum markers allowed us to detect early cardiotoxicity events and provide us an opportunity to start heart failure therapy on time with the aim of improving the control and evolution of it. Levels of miR-133b, miR-21-5p and miR-210 may alert for a risk of cardiotoxicity and can help to make decisions about treatments.

Acknowledgements: Project funded by European Comission (Hecatos FP7-HEALTH-2013-INNOVATION-1. Reference: CP-IP 602156-1) and RETICS program (RD12/0019/0025) cofunded by FEDER "una manera de hacer Europa".
2017 San Antonio Breast Cancer Symposium

Publication Number: P3-14-08

Title: The impact of a structured surveillance protocol using bioimpedance spectroscopy (BIS) on preventing breast cancer related lymphedema (BCRL) in high-Risk patients

Pat Whitworth¹, Andrea Cooper¹, Chirag Shah² and Frank Vicini³. ¹Nashville Breast Center, Nashville, TN; ²Cleveland Clinic, Cleveland, OH and ³Michigan Healthcare Professionals, Farmington Hills, MI.

Body: Background: We evaluated the impact of structured surveillance using bioimpedance spectroscopy (BIS) to prevent clinical BCRL in a group of high-risk (axillary lymph node dissection) patients.

Methods: From April 2010 through November 2016, 93 patients who were treated with axillary lymph node dissection (ALND) were prospectively monitored with BIS using L-Dex (Impedimed). Patients received a pre-operative baseline L-Dex measurement followed by post-operative assessments at regular intervals. An elevated L-Dex score was defined as an increase of ≥10 points above baseline (considered subclinical BCRL). Intervention consisted of applying an over the counter (OTC) sleeve for 4 weeks followed by re-evaluation. The need for complete decongestive physiotherapy (CDP) represented a surrogate for the development of clinically significant, chronic BCRL.

Results: Median follow-up was 24 months (range: 0.3-206.4 months). The median number of nodes removed was 19 (range: 5-41) and the median number of positive nodes was 3. Median age was 53 years old. Eighty percent of patients underwent mastectomy and the remainder breast conserving therapy. 55% of patients received taxane based chemotherapy, 24% received some form of axillary RT (15% high tangents and 9% comprehensive regional nodal RT) and 74% had an elevated body mass index (BMI, > 25). Overall, 75% of these patients had at least one additional high-risk feature, 48% had at least two, and 6% had 3 (either taxane chemotherapy, axillary RT or elevated BMI). Thirty-three patients (35.4%) developed an elevated L-Dex score at some point during follow up. Overall, 10 patients (11%) required CDP at any point after treatment.

Conclusions: The results of this analysis support previously published data on the efficacy of prospective BCRL surveillance and early intervention using BIS. Of the 93 high-risk patients prospectively followed and managed in this structured BCRL protocol, 11% required CDP. These results compare favorably to all contemporary studies reporting BCRL rates in high-risk patients.
Title: Low rates of chronic breast cancer related lymphedema (BCRL) in a cohort of high-Risk patients undergoing prospective surveillance with bioimpedance spectroscopy (BIS)

David Kaufman¹, Chirag Shah² and Frank Vicini³. ¹Breast Cancer Specialists, Bethpage, NY; ²Cleveland Clinic, Cleveland, OH and ³Michigan Healthcare Professionals, Farmington Hills, MI.

Body: Background: We report outcomes using prospective BIS surveillance in a high-risk cohort of patients who all underwent axillary lymph node dissection (ALND).

Methods: From 8/2010 through 12/2016, 206 consecutive patients were evaluated with BIS as part of a prospective surveillance program. 30 underwent ALND and constitute the study population. The program included pre-operative BIS measurement as well as post-operative assessments at regular intervals. Patients with L-Dex readings increasing by more than 10 from baseline were considered to have subclinical BCRL and treated with an over-the-counter (OTC) compression sleeve for 4 weeks. For the purpose of this analysis, additional high-risk features were defined as receipt of axillary radiation, a high body mass index (BMI) or the use of taxane based chemotherapy. Chronic BCRL was defined as the need for complex decongestive physiotherapy (CDP).

Results: Median follow-up was 36 months (range: 4.8-122.1 months). The median number of nodes removed was 18 (range: 5-32) and the median number of positive nodes was two. The median age for the cohort was 57.5 years old with 70% of patients undergoing mastectomy and the remainder breast conserving therapy. With respect to additional high-risk features, 77% also received taxane-based chemotherapy, 62% axillary irradiation, and 48% had an elevated BMI. Overall, 86% of patients had at least one additional high-risk feature, 70% at least two, and 23% had all three additional high-risk features. Seven patients (23%) had an elevated L-Dex score at some point during follow-up and underwent intervention with an OTC sleeve for 4 weeks. To date, no patients have required CDP at any time.

Conclusions: Prospective surveillance with BIS in a high-risk cohort of patients all undergoing ALND (plus additional high-risk features) led to no patients requiring CDP. These excellent findings are consistent with growing data supporting the use of BIS in prospective BCRL surveillance programs.
Title: Proposed biosimilar pegfilgrastim LA-EP2006 shows similarity in pharmacokinetics and pharmacodynamics to reference pegfilgrastim in healthy subjects

Roumen Nakov¹, Sreekanth Gattu¹, Jessie Wang², Maria Velinova³ and Andrej Skerjanec⁴. ¹Hexal AG, Holzkirchen, Germany; ²Sandoz Inc., Princeton, NJ; ³PRA Health Sciences, Gronigen, Netherlands and ⁴Sandoz AG, Basel, Switzerland.

Body: Background: Granulocyte colony-stimulating factors such as filgrastim and its long-acting version pegfilgrastim are widely used to prevent neutropenia in patients receiving chemotherapy. LA-EP2006 is a proposed biosimilar pegfilgrastim that has been compared with reference pegfilgrastim and shown to have no clinically meaningful differences regarding efficacy and safety in breast cancer patients receiving myelosuppressive chemotherapy.¹,²

Methods: This single-dose, randomized, double-blind, two-way crossover study evaluated the pharmacokinetics (PK), pharmacodynamics (PD), immunogenicity and safety of LA-EP2006 and reference pegfilgrastim (Neulasta®, Amgen) in healthy male and female subjects. Subjects were randomized to receive a single 6 mg subcutaneous (sc) injection of LA-EP2006 or reference on Day 1. After dosing, subjects underwent a 4-week assessment period followed by a 4-week washout period before crossing over to receive the other pegfilgrastim and were assessed for a further 4 weeks. The primary objective was to determine PK similarity of LA-EP2006 and reference pegfilgrastim \( (AUC_{0→∞}, AUC_{0→\text{last}} \text{ and } C_{\text{max}}) \), and then PD similarity (absolute neutrophil count [ANC] response in terms of AUEC \( 0→\text{last} \) and \( E_{\text{max}} \)). Secondary objectives included safety and immunogenicity.

Results: A total of 92 subjects were randomized to receive LA-EP2006 then reference (LA-EP2006/reference), and 93 subjects were randomized to receive reference then LA-EP2006 (reference/LA-EP2006) with one subject enrolled but not receiving study medication. A total of 169 subjects were included in the PK and PD analyses. Demographics and baseline characteristics were similar between groups in both treatment periods. PK similarity was shown since the 90% confidence interval (CI) for the geometric mean ratio of LA-EP2006 vs. reference was within the predefined similarity range 0.8–1.25. PD similarity was shown since the 95% CI for the ratio of LA-EP2006 vs. reference was within the predefined similarity range 0.8–1.25 (Table). Secondary endpoints and safety were similar between groups. No neutralising antibodies were detected.

PK and PD parameters for the comparison between LA-EP2006 and reference pegfilgrastim (N=169)*.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Geometric LS means</th>
<th>Point Estimate</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LA-EP2006</td>
<td>Reference</td>
<td>Lower</td>
</tr>
<tr>
<td>PK (serum concentration)</td>
<td>90% CI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( AUC_{0→∞} (\text{ng}\cdot\text{h}/\text{mL}) )</td>
<td>7652</td>
<td>6730</td>
<td>1.1370</td>
</tr>
<tr>
<td>( AUC_{0→\text{last}} (\text{ng}\cdot\text{h}/\text{mL}) )</td>
<td>7487</td>
<td>6574</td>
<td>1.1435</td>
</tr>
<tr>
<td>( C_{\text{max}} (\text{ng}/\text{mL}) )</td>
<td>209</td>
<td>189</td>
<td>1.1082</td>
</tr>
<tr>
<td>PD (ANC)</td>
<td>95% CI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{AUEC}_{0→\text{last}} )</td>
<td>3987</td>
<td>3927</td>
<td>1.0155</td>
</tr>
<tr>
<td>( E_{\text{max}} )</td>
<td>32.6</td>
<td>32.7</td>
<td>0.9951</td>
</tr>
</tbody>
</table>

*N=168 for \( AUC_{0→∞} \); ANC, absolute neutrophil count; \( AUC_{0→∞} \), area under curve measured from the time of dosing and extrapolated to infinity; \( AUC_{0→\text{last}} \), area under curve measured from time of dosing to last measurable concentration; \( \text{AUEC}_{0→\text{last}} \), area under effect curve measured from time of dosing to last measureable concentration; \( C_{\text{max}} \), measured maximum serum concentration after administration; \( E_{\text{max}} \), maximum effect attributable to study drug; PD, pharmacodynamics; PK, pharmacokinetics.

Conclusions: This study shows similar PK, PD and safety of LA-EP2006 to the reference pegfilgrastim.

References:
Title: Comparative effectiveness of antiemetic regimens for highly emetogenic chemotherapy-induced nausea and vomiting: A systematic review and network meta-analysis

Takamichi Yokoe1, Tetsu Hayashida1, Aiko Nagayama1, Tomoko Seki1, Maiko Takahashi1, Toshimi Takano2, Takayuki Abe3 and Yuko Kitagawa1. 1Keio University School of Medicine, 35 Shinanomachi, Shinjyuku, Tokyo, Japan; 2Toranomon Hospital, 2-2-2 Toranomon, Minatoku, Tokyo, Japan and 3Keio University School of Medicine, Clinical and Translational Research Center, 35 Shinanomachi, Shinjyuku, Tokyo, Japan.

Body: Background
The optimal choice of antiemetic therapy for chemotherapy-induced nausea and vomiting (CINV) needs to be clarified. This study assessed the efficacy and safety of antiemetic regimens for highly emetogenic chemotherapy (HEC).

Methods
Randomized trials that compared different antiemetic regimens were included from MEDLINE. Quality was assessed using the Cochrane risk-of-bias tool. We followed Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. Data were pooled using random-effects models. We conducted indirect comparisons using network meta-analysis of a Bayesian model. The main outcomes were the odds ratio (OR) for overall complete response (CR [i.e., no emesis and no rescue]). Safety was assessed from the trial description. All statistical tests were two-sided.

Findings
We systematically reviewed 24 randomized control trials (12,104 participants), which compared 12 different antiemetic regimens. Palonosetron (PAL) 0·75 mg (PAL0·75) + dexamethasone (Dex); aprepitant (APR) + a serotonin-3 receptor antagonist (5HT3) + Dex; and APR + PAL (0·25 mg or 0·50 mg) + Dex were more favorable than the reference regimen (OR, 1·51; 95% credibility interval [95%CrI], 1·18-1·91; OR, 1·78; 95%CrI, 1·58-2·05; and OR, 2·28; 95%CrI, 1·66-3·18, respectively). The oral combination of netupitant and palonosetron (NEPA) was more effective than conventional regimens (OR, 2·39; CrI, 1·73-3·30). Olanzapine (OLZ)-containing regimens were apparently the most effective: the ORs of OLZ + 5HT3 + Dex, OLZ + PAL + Dex, and OLZ + APR + 5HT3 + Dex were 2·78, 2·58, and 4·98, respectively.

Interpretation
The regimens of PAL0·75 + Dex, APR + 5HT3 + Dex, and APR + PAL + Dex were more favorable in conventional regimens (i.e., regimens without NEPA or OLZ), which support the NCCN guideline strategy. NEPA could be a better choice than conventional regimens. OLZ-containing regimens could be an optimal choice; thus, more trials need to be accumulated.
Title: The effect of participation in RCT on outcomes in patients with early breast cancer compared to the general breast cancer population

Meghan B Brennan¹, Dorothy Wiley², Daidong Wang¹, Xiaoyan Wang¹ and Peter Fasching³. ¹UCLA-School of Medicine, Los Angeles, CA; ²UCLA-School of Nursing, Los Angeles, CA and ³University Hospital Erlangen-Fridrich-Alexander-University Erlangen-EMN, Erlangen, Germany.

Body: Background: Breast cancer trial enrollment, while slightly higher than some other histologies, still remains low, at less than 4%. Research suggests lack of clinical trial participation is due to poor patient and clinician commitment and interest. Increasing incentive for trial participation may enhance engagement of patients and healthcare providers leading to increased patient enrollment. One incentive would be to understand benefits of trial participation with regards to better survival and outcomes. The purpose of this study is to determine differences in survival, overall and breast cancer specific, and surgical management, for women who participate in early breast cancer randomized clinical trials (RCT) compared to the general breast cancer population who received similar standard therapy outside of a RCT.

Methods: Patients included in this retrospective analysis were from one of three (3) international, randomize, adjuvant breast cancer trials (RCT-participants) and women with breast cancer from the general U.S. population, from Surveillance Epidemiology and End Results Program (SEER-13), the controls. Women diagnosed between 1997-2004 with invasive breast cancer, tumor (T) size 1-3, lymph node (LN) positive (LN1/2), hormone receptor positive or negative, HER2 positive or negative, treated with surgery, adjuvant radiation, and chemotherapy were included in the analysis. In this study, propensity score analysis (PSA) was done to provide weight to each data point for each variable in order to more closely represent similar populations. PSA, considered superior to a standard Cox multivariate analysis as it attempts to reduce the bias due to confounding or correlated predictive variables. Subsequently, the propensity score was applied to a Cox proportional hazards model to determine hazard ratios (HR), with a Wald 95% confidence interval (CI), of trial participation on survival. Similarly, PSA was done for surgical outcomes. A multivariate logistic regression was performed to calculate the odds ratios, with a Wald 95% CI, to determine if RCT participation compared to the SEER-13 control had an impact on surgical outcomes, mastectomy versus breast conserving surgery (BCS).

Results: The total sample size was 9255 patients, 1795 RCT-participants and 7460 SEER-13 controls. After controlling for all other significant predictors of survival, RCT participation significantly reduced risk of breast cancer related death at 5-years by more than 25% and 18% at 10 years [HR: 0.75 (95% CI: 0.64-0.87); p=0.00020; and HR: 0.83 (95% CI: 0.74-0.93); p=0.00165, respectively]. Additionally, we demonstrated a significant reduction in risk of all-cause mortality for RCT-participants, at both 5-years and 10-years [HR: 0.83 (95% CI: 0.72--0.95); p=0.009; and HR: 0.79 (95% CI: 0.71-0.87); p<0.00001, respectively]. Additionally, RCT-participants were significantly less likely to undergo invasive surgical management (mastectomy) compared to SEER-13 controls [OR: 0.78 (95% CI: 0.66-0.92;) p=0.03].

Conclusion: RCT-participants have a reduced risk of death at 5 years and 10 years compared to the general breast cancer population. Additionally, RCT-participants are less likely to undergo mastectomy than the SEER-13 controls.
Treat ER+ight Canadian prospective observational study in HR+ advanced breast cancer: 2nd interim analysis

Susan Dent¹, Nadia Califaretti², Catherine Doyle³, Cristiano Ferrario⁴, Edmond Chouinard⁵, Swati Kulkarni⁶, Josee-Anne Roy⁷, Sabrina R Perri⁸ and Stephen Chia⁹. ¹Ottawa Hospital Cancer Center, Ottawa, ON, Canada; ²Grand River Regional Cancer Center, Kitchener, ON, Canada; ³Deschênes-Fabia Breast Cancer Center, Quebec City, QC, Canada; ⁴Segal Cancer Center - Jewish General Hospital, Montreal, QC, Canada; ⁵Cambridge Memorial Hospital, Cambridge, ON; ⁶Windsor Regional Cancer Center, Windsor, ON, Canada; ⁷Hopital Sacré-Coeur de Montreal, Montreal, QC, Canada; ⁸Novartis Pharmaceuticals Canada Inc., Dorval, QC, Canada and ⁹BC Cancer Agency - Vancouver Cancer Center, Vancouver, BC, Canada.

Body: Treat ER+ight is the 1st prospective observational study in Canadian postmenopausal women with HR+ HER2– advanced breast cancer currently receiving endocrine therapy (ET) alone or in combination with targeted therapy (TT) (NCT02753686).

Methods: This pre-planned interim analysis describes baseline characteristics, treatment sequence, monitoring patterns, patient-reported quality-of-life (QoL) and resource utilization of patients enrolled in ET and ET+TT cohorts within the 1st 3 months of therapy. At data cut-off (13Mar‘17), 100 patients were enrolled from 24 sites since Mar‘16.

Results:

Baseline Patient and Disease Characteristics

<table>
<thead>
<tr>
<th></th>
<th>ET (n=42)</th>
<th>ET + TT (n=58)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age, years (range)</td>
<td>70 (37-88)</td>
<td>63 (39-80)</td>
</tr>
<tr>
<td>ECOG 0-1, (%)</td>
<td>60</td>
<td>72</td>
</tr>
<tr>
<td>Median time since primary BC diagnosis, years (range)</td>
<td>4.5 (0-37)</td>
<td>5 (0-25)</td>
</tr>
<tr>
<td>Median time with advanced BC diagnosis, years (range)</td>
<td>1 (0-16)</td>
<td>1 (0-7)</td>
</tr>
<tr>
<td>Sites of metastases (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone only</td>
<td>38</td>
<td>29</td>
</tr>
<tr>
<td>Visceral only</td>
<td>33</td>
<td>38</td>
</tr>
<tr>
<td>Bone + visceral</td>
<td>29</td>
<td>24</td>
</tr>
<tr>
<td>Last prior line of therapy included but not limited to (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Letrozole</td>
<td>41</td>
<td>31</td>
</tr>
<tr>
<td>Anastrozole</td>
<td>12</td>
<td>19</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>Exemestane</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>Palbociclib+Fulvestrant</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Line (L) of metastatic therapy (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1L</td>
<td>19</td>
<td>22</td>
</tr>
<tr>
<td>2L</td>
<td>38</td>
<td>43</td>
</tr>
<tr>
<td>3L</td>
<td>43</td>
<td>35</td>
</tr>
</tbody>
</table>

Enrollment therapy (%): everolimus+exemestane (35), fulvestrant (15), palbociclib + letrozole (15), tamoxifen (14), exemestane (7), palbociclib+fulvestrant (7), letrozole (4) and palbociclib+exemestane (1). Follow-up visits with physician after therapy initiation ET, ET+TT (%): week 2 (5, 47), month 1 (71, 67), month 3 (43, 31). Numerical differences were observed in EORTC QLQ C30 and BR23 global health status/QoL, functional and symptom scale scores between ET and ET+TT cohorts. Mean changes in
transformed scores from baseline to month 3 were generally similar between cohorts however 'therapy side effects' symptom item worsened in ET+TT cohort (P = 0.031, Wilcoxon rank sum). Resource utilization in between scheduled visits during 1st 3 months of therapy was similar between cohorts for physician/radiology, hospitalizations and ER visits however patient calls to supportive care nurse was increased in ET+TT cohort (P = 0.008, Fisher’s exact). Treatment discontinuation rate (20%) was similar between cohorts with progression as predominant reason for initiating next therapy. Chemotherapy (CT) was the most frequent subsequent therapy and represented 1st line metastatic CT for majority of patients.

Conclusions:
ET+TT patients were younger, had better ECOG, more visceral disease and 60% received everolimus+exemestane with <10% receiving prior CDK4/6 therapy. Half of patients in ET+TT cohort returned for a follow-up visit with oncologist within 2 weeks of therapy initiation (compared to minority in ET cohort) and called the supportive care nurse in between scheduled visits. Patient-reported Qol within 1st 3 months of therapy was generally similar between cohorts. Therapy-related AEs impacted the patient-reported experience in ET+TT cohort however AEs did not lead to therapy discontinuation in over 85% of cases. These results highlight the importance of: (1) proactive AE patient education/management upon ET+TT initiation and at subsequent follow-up visits, (2) week 2 visit and (3) additional nursing telephone support in between visits.
2017 San Antonio Breast Cancer Symposium

Publication Number: P3-15-03

Title: Clinical benefit, toxicity, and cost of metastatic breast cancer drug therapies: Visualizing randomized and non-randomized evidence

John Silberholz¹, Linda Vahdat² and Dimitris Bertsimas³. ¹University of Michigan, Ann Arbor, MI; ²Weill Cornell Medicine, New York, NY and ³Massachusetts Institute of Technology, Cambridge, MA.

Body: Background: Given the rapidly expanding size of the clinical trial literature for metastatic breast cancer (MBC) clinical trials, oncologists need tools that enable them to efficiently review the settings and results of previous studies.

Methods: We searched the Cochrane Central Register of Controlled Trials and PubMed to identify clinical trials testing MBC drug therapies. Key eligibility criteria included >90% of patients enrolled in trial having MBC, therapeutic clinical trials, and Phase II or III studies with at least 10 patients at a dosage level. From each study, we extracted information about clinical benefit and toxicity of drug therapies, and we further estimated the costs of all drug therapies. We built a web-based visualization tool that presents the results of all studies testing a given drug combination, stratified based on patients’ receptor statuses and prior exposure to therapy. The tool presents both aggregate and study-level results, and reports both unadjusted study results and standardized results based on key patient characteristics in the study populations.

Results: We included 1,783 studies containing 2,549 treatment arms and 170,515 patients in the visualization tool. The tool reports information on 77 HER-2-directed therapies, 101 hormonal therapies, and 675 undirected therapies. The visualization tool has been made publicly accessible for patients and doctors to access at http://cancertrials.info.

Discussion: We believe this tool will make literature reviews significantly more efficient for oncologists, enabling them to better leverage insights from the clinical trial literature when making treatment decisions.
**Title:** Signalling pathways targeted by the YangZheng Xiaoji extract and the therapeutic implications in human breast cancer

Wen G Jiang¹, Lin Ye¹, Sioned Owen¹, Fiona Ruge¹, Tracey A Martin¹, Andrew J Sanders¹, Gong Gao², Cong Wei², Yiling Wu² and Eleri Davies³. ¹Cardiff University School of Medicine, Cardiff, Wales, United Kingdom; ²Yiling Pharmaceuticals, Cardiff, Hebei Province, China and ³Breast Cancer Centre, University Llandough Hospital, Cardiff, Wales, United Kingdom.

**Body:** Background. Yangzheng Xiaoji is a formulation of Chinese medicine and has been used in the treatment of solid cancer as an adjuvant to chemotherapy by reducing the side effects to the patient. There has been evidence to show that the medicine has a direct biological role in cancer cells. In the present study, we sought to investigate the potential effects of the medicine on breast cancer cells and in particular aimed to identify the key targets and molecular pathways contributing to the anti-cancer effect of the medicine.

Methods. Human breast cancer cell lines (BT549, BT20, MDA MB-231, MCF-7 and ZR 75-1) with varying invasiveness and receptor status were used. The soluble extract of Yangzheng Xiaoji, namely DME25 was used in the study. The effects of DME25 on the growth, toxicity and cellular migration were assessed. Signalling kinase changes were screened using kinase antibody array based array technologies. Kinases were also validated using phosphorylation based protein blotting.

Results. Of the five breast cancer cell lines tested, Yangzheng Xiaoji extract DME25 showed little cytotoxicity over a broad range of concentrations. However, DME25 were able to markedly reduce the migration of the panel of breast cancer tested, without being toxic. Triple negative cells responded in a similar fashion with other cells. It was also noted that the adhesion of these cells were also inhibited by DME25. Using a protein kinase array, it was shown that a number of kinase complexes were inhibited by the medicine, notable ones including EGFR family kinases (reduced by 35%), Janus protein kinases (JAK) (by 57%), and Ras-related C3 botulinum toxin substrate (Rac1 or CDC42 GTPase) (by 49%) and Ribosomal protein S6 kinases (RSKs) (by 52%). Given the clinical significance of RSKs in human breast cancer, we further evaluated the role of RSK and RSK inhibitors in DME mediated cell functions and have demonstrated that both in triple negative breast cancer cells and receptor positive breast cancer cell lines, DME25 was able to synergistically enhance the effect of RSK2 inhibitor, SL1010-1, on the both the cellular migration and cell growth.

Conclusion. Yangzheng Xiaoji has a broad and direct effect on the migration of breast cancer, an effect unrelated to hormone receptor status and independent of cytotoxicity. The medicine appears to target kinase pathway, particularly for the RSK kinases, suggesting an important clinical implication in the treatment of breast cancer.
Title: Early breast cancer in the elderly: characteristics, therapy, and long-term outcome

Omer Gal¹, Yael Ishai¹, Aaron Sulkes¹,³, Tzipora Shochat² and Rinat Yerushalmi¹,³. ¹Davidoff Cancer Center, Rabin Medical Center - Beilinson Hospital, Petach Tikva, Israel; ²Statistical Consulting Unit, Rabin Medical Center - Beilinson Hospital, Petach Tikva, Israel and ³Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

Body: Objective: The number of older adults diagnosed with breast cancer is increasing. However, data on breast cancer characteristics, treatment, and survival in elderly women are sparse.

Methods: The database of a tertiary cancer center was searched for all women aged ≥65 years who were diagnosed with early breast cancer in 2004-2007. Patients were divided into two age groups: 65-75 years and >75 years. Data on tumor, treatment and outcome parameters were compared.

Results: The cohort included 390 patients. The older group underwent more mastectomies but less axillary surgery or adjuvant systemic therapy. Median overall survival (OS) was 9.5 years in the older group and not reached in the younger group; the 8 year disease-free survival rates were 85% and 88%, respectively (p=0.27). Both age and tumor subtype had an effect on OS and recurrence rates (p<0.001 for OS; p=0.16 for recurrence). The worst outcome was noted in women aged >75 years with triple-negative (TN) disease.

Conclusion: Treatment approach was different between both age groups, despite similar tumor characteristics. TN subtype presented as the most aggressive disease in both age groups. Physicians should be alert to these findings and select treatment on a case-by-case basis.
Title: Using the new pan-cancer clinical data resource (TCGA-CDR) to identify breast cancer genomic correlates associating with different survival outcome endpoints

Jianfang Liu¹, Tara Lichtenberg², Katherine A Hoadley³, Andrew Cherniack⁴, Laila Poisson⁵, Albert J Kovatich⁶, Christopher Benz⁷, Vesteinn Thorsson⁸, TCGA PanCanAtlas Research Network, Craig D Shriver⁹ and Hai Hu¹.¹ Chan Soon-Shiong Institute of Molecular Medicine at Windber, Windber, PA; ²Nationwide Children's Hospital, Columbus, OH; ³University of North Carolina at Chapel Hill, Chapel Hill, NC; ⁴The Eli and Edythe L. Broad Institute of MIT and Harvard, Cambridge, MA; ⁵Henry Ford Health System, Detroit, MI; ⁶Clinical Breast Care Project, Murtha Cancer Center, Uniformed Services University / Walter Reed National Military Medical Center, Bethesda, MD; ⁷Buck Institute for Research on Aging, Novato, CA; ⁸Institute for Systems Biology, Seattle, WA and ⁹Murtha Cancer Center, Uniformed Services University / Walter Reed National Military Medical Center, Bethesda, MD.

Body: Introduction

The Cancer Genome Atlas (TCGA) generated abundant high quality molecular data, however its relatively short-term patient follow-up limited its immediate clinical utility. We led a PanCanAtlas effort to systematically collate, integrate, and quality check the large body of acquired clinicopathologic data, generated 4 primary clinical outcome endpoints for each case, and created a new Pan-Cancer Clinical Data Resource (TCGA-CDR) for public use. We report here on the utility and validity of this TCGA-CDR in relating breast cancer (BC) genomic information to survival endpoints.

Methods

Clinicopathologic data from all data files were integrated and processed. Overall survival (OS), disease-specific survival (DSS, an approximation), progression-free interval (PFI), and disease-free interval (DFI) were derived. Tests of the adequacy of the follow-up intervals for each endpoint were performed, and quality evaluation of these endpoints was established by their comparison with different clinical features. As a case study we compared each survival endpoint for significant association (FDR <0.2) with chromosomal aneuploidy.

Results

The 4 endpoints were derived for 1097 TCGA BC cases having a median follow-up time of 27.7 months. Median times to events/censorship for OS, DSS, PFI, and DFI were 41.8/25.0, 32.6/26.0, 26.0/25.0, and 25.4/25.0 months respectively. PFI and DFI passed tests for adequate follow-up times; OS and DSS partially passed the same tests signaling some caution with their use in genomic associations.

Using the endpoints, outcomes of patients with ER+ and ER- tumors were compared, along with those of patients with low (I&II) and high (III&IV) stage breast tumors. Univariate analyses suggested patients with ER+ tumors had significantly better survival than patients with ER- tumors when using PFI (p=0.005), DFI (p=0.001), and DSS (p=0.009), with OS not reaching significance (p=0.09). Patients with low stage tumors showed significantly better outcomes than patients with high stage tumors for each endpoint (p<0.001). The 4 endpoints were also evaluated for their significant associations with chromosomal arm aneuploidy. Adjusted for patient age and AJCC stage, tumors with a loss of 8q and 8p (p=0.019, FDR=0.37) had worse PFI; and those with loss of 8q, 20q, and 8p had worse DFI. Tumors with gain of 11q or loss of 14, 7q, 12q, 18q, 20q, 3p, 7p, 8p, 18p, and 20p had worse OS. In contrast, tumors with loss of 16q had better DSS, while those with loss of 3q, 12q, 17q, 18q, 19q, 20q, 3p, 8p, 12p, 18p, 19p, and 20p had worse DSS. The finding that 8p loss associated with worse survival for all 4 endpoints, while 18p loss associated with worse OS and DSS, agrees with literature reports.

Conclusion

These findings confirm that PFI and DFI, as extracted from the TCGA-CDR, are valid and appropriate BC survival endpoints, while OS and DSS may be recommended with some caution when employing TCGA data to evaluate new relationships between breast cancer genomic abnormalities and clinical outcomes.

The views expressed in this article are those of the author and do not reflect the official policy of the Department of Army/Navy/Air Force, the Department of Defense, or U.S. Government.
Title: Geographic variation in adverse event reporting patterns in breast cancer clinical trials

Valeria González1, Andrés Machado1, Helena Fung2, Gonzalo Spera1, Carlos Meyer1, Pablo Millán1, John R Mackey3 and Rodrigo Fresco1. 1Translational Research in Oncology (TRIO), Montevideo, Uruguay; 2Translational Research in Oncology (TRIO), Edmonton, AB, Canada and 3University of Alberta, Edmonton, AB, Canada.

Body: Introduction: Adverse event (AE) reporting in clinical trials (CT) informs the safety of investigational products. Once approved, safety information in the monograph and prescribing information mainly derive from CT data. Some studies have shown geographic variations in the AE reporting patterns in multinational CT; none of them assessed this variation in cancer CT. We conducted a study to analyze the geographic AE reporting patterns in two breast cancer (BC) CT conducted by Translational Research in Oncology (TRIO).

Objective: To perform a quantitative and qualitative comparison of non-serious AE (NSAE) and serious AE (SAE) reporting patterns between several geographic regions, in breast cancer CT conducted by TRIO.

Methodology: We retrospectively analyzed aggregated NSAE/SAE data (as reported by investigators) from all patients randomized in two completed phase 3, multinational CT of anticancer therapies in advanced BC. Participating countries were grouped in 7 regions according to their geographic location (East Asia, Eastern Europe, Latin America and Caribbean, Middle East and Africa, Non-Eastern Europe, North America, Oceania). Regions were kept masked and numbered from 1 thru 7. AE data were extracted from the clinical data bases. For each region we calculated the mean number of NSAE and SAE per patient (pt), the mean number of NSAE and SAE per cycle/per pt, and the percentage (%) of pt experiencing selected AE (fatigue, febrile neutropenia and emesis). Comparisons between regions were done using unequal variance t-test and Fisher’s exact test.

Results: 1,863 patients from 35 countries and 310 sites were included. Mean number of pt per region was 331. We found significant variation in the number of NSAE/SAE reported across several regions. Two regions (1 and 6) reported the highest mean number of AE while region 4 the lowest rates. The mean number of NSAE reported in region 4 is approximately 3-fold lower than regions 1 and 6 (mean NSAE 22.8 [region 1] vs. 7.9 [region 4]; p <.0001; mean NSAE cycle/pt 9.7 [region 1] vs. 3.2 [region 4]; p <.0001). Region 4 reported 8-fold lower rates of SAE than region 1 (mean number SAE 0.1 vs. 0.8, p<.0001) (Table 1). % of pt experiencing AE fatigue, febrile neutropenia and nausea/vomiting also varied significantly across regions, especially between regions 1 and 4 (Table 2).

<table>
<thead>
<tr>
<th>NSAE and SAE reporting in selected regions (regions 1 and 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Region 1 (mean)</td>
</tr>
<tr>
<td>NSAE per pt</td>
</tr>
<tr>
<td>SAE per pt</td>
</tr>
<tr>
<td>NSAE per cycle/per pt</td>
</tr>
<tr>
<td>SAE per cycle/per pt</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>% of pt experiencing selected AE in selected regions (regions 1 and 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Region 1</td>
</tr>
<tr>
<td>Fatigue</td>
</tr>
<tr>
<td>Febrile Neutropenia</td>
</tr>
<tr>
<td>Nausea and Vomiting</td>
</tr>
</tbody>
</table>
Conclusion: NSAE and SAE reporting patterns vary markedly by geographic region and one region appears to systematically under report both NSAE and SAE. These data warrant confirmation, and if confirmed, may provide an important caveat on the interpretation of reported study safety data.
Title: Influence of non-measurable disease on progression-free survival in patients with metastatic breast cancer

Hadar Goldvaser¹, Domen Ribnikar¹, Rouhi Fazelzad¹, Bostjan Seruga², Arnoud J Templeton³, Alberto Ocana⁴ and Eitan Amir⁴.
¹Princess Margaret Cancer Centre, Toronto, ON, Canada; ²Institute of Oncology Ljubljana, Slovenia, Ljubljana, Slovenia; ³St. Claraspital Basel and Faculty of Medicine, University of Basel, Basel, Switzerland and ⁴5Research Unit. Albacete University Hospital, Albacete, Spain.

Body: Background:
Progression free-survival (PFS) is the dominant endpoint in phase 3 randomized controlled trials (RCTs) in women with metastatic breast cancer (MBC), and requires the ability to measure target lesions. It is unknown whether treatment effect on PFS is consistent among patients with measurable and non-measurable disease.

Methods:
We searched MEDLINE, EMBASE and COCHRANE for phase 3 RCTs in MBC that reported outcomes in subgroups with non-measurable (or bone only disease, if not reported explicitly) and measurable disease. Data were extracted and a single hazard ratio (HR) and 95% confidence intervals (CI) were computed to compare the individual trial treatment effect in non-measurable versus measurable disease. Data were then pooled in a meta-analysis. We repeated the analysis comparing bone only to non-bone only disease and performed subgroup analyses based on drug mechanism of action.

Results:
Of 82 RCTs that enrolled patients with non-measurable disease, 16 trials comprising 8516 patients were eligible for analysis. All included RCTs used PFS or time to progression as primary endpoints. There was no difference in pooled treatment effect between patients with non-measurable and measurable disease (HR 1.01, 95% CI 0.89-1.15, p=0.82). However, compared to non-bone only disease, a significantly greater effect on PFS was seen in those with bone only disease (HR 0.82, 95% CI 0.70-0.98, p=0.03). Subgroups analyses according to drug mechanism are shown in Table 1

<table>
<thead>
<tr>
<th>Cohort/ Investigational drug</th>
<th>No. studies included</th>
<th>Measurable HR (95% CI)</th>
<th>Non measurable HR (95% CI)</th>
<th>Intra- study comparison HR (95% CI)</th>
<th>P – for intra-study comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>16</td>
<td>0.69 (0.65-0.73)</td>
<td>0.72 (0.64-0.80)</td>
<td>1.01 (0.89-1.15)</td>
<td>0.82</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>3</td>
<td>0.99 (0.87-1.13)</td>
<td>0.67 (0.44-1.02)</td>
<td>0.73 (0.44-1.21)</td>
<td>0.22</td>
</tr>
<tr>
<td>Endocrine treatment</td>
<td>4</td>
<td>0.86 (0.77-0.96)</td>
<td>0.94 (0.80-1.10)</td>
<td>1.13 (0.92-1.40)</td>
<td>0.23</td>
</tr>
<tr>
<td>Signal transduction inhibitors</td>
<td>4</td>
<td>0.52 (0.48-0.57)</td>
<td>0.41 (0.33-0.50)</td>
<td>0.74 (0.59-0.94)</td>
<td>0.01</td>
</tr>
<tr>
<td>Anti-angiogenetic agents</td>
<td>5</td>
<td>0.66 (0.59-0.73)</td>
<td>0.84 (0.67-1.04)</td>
<td>1.34 (1.05-1.71)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

CI- confidence interval, HR- hazard ratio

. Compared to patients with measurable disease, there was a greater effect on PFS in those with non-measurable disease in RCTs of signal transduction inhibitors and endocrine therapy (HR 0.74, 95% CI 0.59-0.94, p=0.01). There was a lesser effect on PFS in patients with non-measurable disease in RCTs of antiangiogenic drugs (HR 1.34, 95% CI 1.05-1.71, p=0.02). Comparable effect on PFS was shown in RCTs evaluating endocrine therapy and chemotherapy.

Conclusions:
There is variability in treatment effect on PFS in patients with measurable and non-measurable disease. There is greater effect on PFS in RCTs of endocrine therapy and signal transduction inhibitors and in patients with bone only disease. Standardization of PFS determination in patients with non-measurable and bone only disease is warranted.
**Title:** Concordance of real world progression free survival (PFS) on endocrine therapy as first line treatment for metastatic breast cancer using electronic health record with proper quality control versus conventional PFS from a phase 3 trial

Cynthia Huang Bartlett¹, Jack Mardekian¹, Matthew Cotter¹, Xin Huang¹, Zhe Zhang¹, Christina M Parrinello², Amy P Abernethy² and Maria Koehler¹. ¹Pfizer, New York, NY and ²Flatiron Health, New York, NY.

**Body:**

**Background:** There is growing interest to assess treatment effect with clinical endpoints that are reflective of real world clinical practice based on data systemically collected from the electronic health record (EHR). This study compared real world PFS (rwPFS) among a cohort of female patients (pts) treated with letrozole (LET) monotherapy as first-line therapy for metastatic breast cancer (MBC) in routine clinical practice to PFS using RECIST criteria from the LET alone cohort in a randomized clinical trial (PALOMA-2).

**Methods:** For rwPFS, assessments were based on clinician notes, radiological reports, and pathological reports available in the EHR. Data were extracted from the EHR using technology-enabled abstraction; relevant documents were presented to trained human experts and data were entered into an electronic interface mimicking a case report form, with centralized management and quality controls. The patient cohort was derived from a large, longitudinal, demographically and geographically diverse EHR database of pts receiving cancer care at U.S. community-based and academic clinics (Flatiron Health); pts were all females who began treatment with LET for MBC as first-line treatment between January 1, 2011 and September 30, 2015. PFS in the clinical trial used a conventional definition based on RECIST; data were from the Pfizer clinical study PALOMA-2 database; 222 post-menopausal women in the LET alone arm met pre-specified inclusion/exclusion criteria and assessment intervals with enhanced data collection as previously detailed (Finn, NEJM 2016). All data were de-identified. Propensity score matching using a nearest neighbor algorithm with caliper=0.10 (maximum difference in propensity scores for a match) was performed to assess the consistency between the two approaches to PFS assessment. Kaplan-Meier estimates of median rwPFS/PFS are reported.

**Results:** 107 pts met eligibility criteria from the EHR subset. Pts were younger and had less extensive disease in the PALOMA 2 cohort: The mean age was 60.6 vs 68.6 years and the proportion stage IV at diagnosis was 32.4% versus 39.3% in the LET alone cohort in PALOMA-2 compared to the EHR cohort, respectively. Propensity matching with caliper=0.10 based on age, stage at diagnosis and # of disease sites identified 79 pts from each cohort.

**Summary of Progression-Free Survival in Matched Data Using Propensity Score Matching Method (Kaplan-Meier estimates)**

<table>
<thead>
<tr>
<th>Source</th>
<th>rwPFS</th>
<th>RECIST PFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unmatched results</td>
<td>Flatiron</td>
<td>PALOMA-2</td>
</tr>
<tr>
<td>N (pts)</td>
<td>107</td>
<td>222</td>
</tr>
<tr>
<td>median PFS (months)</td>
<td>18.7</td>
<td>14.5</td>
</tr>
<tr>
<td>95% Confidence Interval</td>
<td>(14.6, 24.1)</td>
<td>(12.9, 17.1)</td>
</tr>
<tr>
<td>Matching with Caliper (=0.10)</td>
<td>N (pts)</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>median PFS (months)</td>
<td>18.5</td>
</tr>
<tr>
<td></td>
<td>95% Confidence Interval</td>
<td>(13.7, 24.1)</td>
</tr>
</tbody>
</table>

**Conclusion:** This analysis showed concordance between rwPFS and RECIST-based PFS for LET-treated MBC matched pts using a caliper=0.10 in the first line setting. Clinically meaningful information on treatment effect could be derived from a rwPFS assessment based on EHR data abstraction that incorporates proper quality controls.
Impact: These data increase confidence in the use of rwPFS as a primary outcome in real-world studies.
Title: Adiposity at age 10 and mammographic density among premenopausal women

Aliya Alimujiang1, Kellie R Imm1, Catherine M Appleton7, Graham A Colditz1, Catherine S Berkey3 and Adetunji T Toriola1.
1Washington University School of Medicine and Siteman Cancer Center, St Louis, MO; 2Washington University School of Medicine, St Louis, MO and 3Brigham & Women’s Hospital and Harvard Medical School, Boston, MA.

Body: Background: Higher mammographic breast density is a strong risk factor for breast cancer. Early-life factors may influence breast development and subsequent breast density in adulthood. Although childhood adiposity is inversely associated with breast cancer risk, the association of childhood adiposity with mammographic breast density in premenopausal women has not been adequately studied. This knowledge could provide insight into pathways linking mammographic density and breast cancer risk. We investigated the associations between adiposity at age 10 and mammographic density.

Methods: We collected data from 370 premenopausal women during their routine screening mammograms at Washington University in St. Louis, MO from December 2015 to October 2016. Body size at age 10 was self-reported using the Stunkard 9-figure Somatotype pictogram. For these analyses, the Stunkard pictogram was collapsed into 4 groups: (i) body size 1 or 2, (ii) body size 3 or 4, (iii) body size 5, (iv) body size 6 or higher. Body mass index (BMI) at age 10 was imputed using BMI and Stunkard data from the Growing Up Today Study. Trained personnel collected women’s height and weight, which were used to calculate current BMI. We used the Volpara software to evaluate the following volumetric mammographic density measures: volumetric percent density (VPD), dense volume (DV) and non-dense volume (NDV). Age-adjusted Pearson correlations and multivariable linear regression models (adjusted for age, age at each given birth, family history, race, education, oral contraceptive use, and breast feeding history) were used to evaluate the associations between adiposity at age 10 and volumetric mammographic density measures.

Results: The mean age at the time of screening mammogram was 47.1 years. The majority of the women (43.8%) reported having body size 1 or 2, followed by body size 3 or 4 (34.9%), body size 5 (13.8%) and body size 6 (7.6%) at age 10. We observed a negative correlation between BMI at age 10 ($r = -0.28$, p-value <0.001) and VPD, and a positive correlation between BMI at age 10 ($r = 0.27$, p-value <0.001) and NDV. In multivariable regression models, adiposity at age 10 was significantly inversely associated with VPD, and positively associated with NDV. A 1 kg/m$^2$ increase in BMI at age 10 was associated with a 6.3% decrease in VPD (p-value <0.001), and a 6.7% increase in NDV (p-value <0.001). Compared to women whose body sizes were 1 and 2 at age 10, women with body size 3 or 4 had a 17.6% decrease in VPD, and a 28.5% increase in NDV; women with body size 5 had a 32.3% decrease in VPD, and a 58.1% increase in NDV, and women with body sizes ≥6 had a 46.6% decrease in VPD, and a 75.1% increase in NDV (all p-values <0.05). The associations of body size at age 10 and VPD were attenuated, but still statistically significant when we adjusted for current BMI. No statistically significant associations were found between adiposity at age 10 and DV.

Conclusions: Our findings of an inverse association between adiposity at age 10 and percent density suggest that adiposity at age 10 could impact breast cancer development via its effect on mammographic density. Mechanistic studies to understand how childhood adiposity reduces mammographic density and breast cancer development in premenopausal women are needed.
Title: Choosing wisely: Radiographic surveillance after breast conservation therapy in HER2+ breast cancer

Ashlyn Everett¹, Audrey S Wallace¹, Jennifer F De Los Santos¹, Gabrielle B Rocgue¹, Catherine S Parker¹ and Kimberly S Keene¹. ¹University of Alabama Birmingham.

Body: Purpose/Objective(s): ASTRO Choosing Wisely campaign recommends no more than annual mammography after breast conservation therapy (BCT). HER2+ disease portends increased risk of locoregional recurrence in comparison to Luminal A disease. Our previous institutional practice included surveillance imaging every 6 months for the first 2-3 years after BCT. The purpose of this study is to evaluate surveillance imaging intervals as a means of detecting early locoregional recurrence at one institution.

Materials/Methods: Women with HER2+ locoregionally confined invasive breast cancer treated with lumpectomy and radiation as part of breast conservation therapy at one institution were retrospectively identified after IRB approval. Patient demographics, treatment, surveillance, and outcomes data were captured. BRCA+ patients or those without available surveillance follow-up at our institution were excluded. Surveillance period started after the last fraction of radiation was completed.

Results: In 86 women treated from 2008-2016, median age at diagnosis was 57, and 69% were Caucasian. Most patients were treated for Stage I-II invasive ductal carcinoma. All but one were treated with chemotherapy, with similar distribution between adjuvant and neoadjuvant regimens. Receptor status was as follows: ER+ 66%, PR+ 59%, ER+/PR+/HER2+ in 58%, ER-/PR-/HER2+ in 33%. Mammography +/- ultrasound was utilized, with infrequent use of magnetic resonance imaging. Median time from end of radiation to first surveillance imaging was 4 months. All women had 1 post-BCT imaging, with 2nd, 3rd, and 4th available in 91%, 85%, 73% respectively. Interval frequency between first 4 images was 6 months. No patients had expired at the time of analysis. Recurrence were identified in 2 women: 1 identified clinically after negative mammography, and 1 identified on mammography at 27 months.

Conclusion: While overall events are small, discordant biannual mammography did not identify locoregional recurrences in this biologically higher risk group in the first two years post-treatment. This data supports current guideline recommendations limiting surveillance breast imaging in patients undergoing breast conservation to an annual frequency.
Recall rates during breast cancer surveillance in high-risk women with dynamic contrast-enhanced magnetic resonance imaging every 6 months: Results from a single institution study

Kristen D Whitaker¹, Hiroyuki Abe¹, Deepa Sheth¹, Dezheng Huo¹, Toshio F Yoshimatsu¹, Marion Verp¹, Yonglan Zheng¹, Greg Karczmar¹, Rodrigo Guindalini¹ and Olufunmilayo I Olopade¹. ¹University of Chicago, Chicago, IL.

Purpose: To compare recall rates and biopsy rates in high-risk women undergoing semi-annual dynamic contrast-enhanced magnetic resonance imaging compared to recommended annual DCE-MRI.

Background: In high-risk women with BRCA1/BRCA2 mutations and/or a personal or family history of breast cancer, annual breast MRI has shown improved sensitivity and cancer detection compared to mammography and is recommended annually in addition to mammogram. The routine use of breast MRI screening in this patient population is not widespread due to concerns for higher recall rates and false positive biopsy results, which often contribute to higher healthcare costs and increased stress. Breast MRI screening acceptability is dependent on sensitivity and recall rates. The acceptable recall rate for breast MRI is generally considered to be between 6-12% based off the results from studies that used annual DCE-MRI screening.

Methods: Between 2004 and 2016, a prospective cohort of high-risk women underwent semi-annual DCE-MRI and annual mammography. For subjects with BI-RADS score of 4 or 5 on DCE-MRI, biopsy was recommended. For subjects with BI-RADS score of 0 on DCE-MRI and/or BI-RADS scores of 0,4, or 5 on MG, further investigation by imaging was recommended and biopsy was performed if clinically appropriate. Tests with BI-RADS scores of 3 were discussed case-by-case. Recall was defined as women being recommended for further imaging (i.e. US and/or MG) in order to provide additional information. Women that are recalled may go on to have a subsequent biopsy based on the findings.

Results: 295 women were recruited to the study; 44% of the study participants had mutations in BRCA1 or BRCA2. 2111 DCE-MRI screening tests and 1225 mammography were performed. The sensitivity and specificity was 93.7% and 96.6% respectively for DCE-MRI and 50% and 97.7% respectively for mammogram. The positive predictive value was 17% for MRI and 22% for mammography. Eighty-nine women had 106 recalls. 74 due to DCE-MRI imaging alone, 18 due to mammography alone, and 14 due to both image modalities. The recall rate was 4.2% for DCE-MRI and 2.6% for mammography. In total, 56 biopsies were performed. 3 DCIS and 13 invasive breast cancers were diagnosed. On average, 5.9 women would have to be recalled on DCE-MRI with 3.3 biopsies to diagnose one cancer case. 4 women would have to be recalled on mammography with 2.1 biopsies to diagnose one cancer.

Conclusion: Semi-annual DCE-MRI screening in high-risk women demonstrated high sensitivity without substantially increasing recall rates or biopsy rates to an unacceptable value. Our single institution DCE MRI protocol achieved recall rates lower than those considered acceptable for annual MRI or mammography. This study demonstrates that with radiology reader expertise, careful clinical decision making, and improved MRI technology, it is possible to achieve recall rates lower than those achieved with annual mammography or MRI even when DCE-MRI screening exams occur more frequently than those currently recommended by guidelines. Additional data including QOL and cost effectiveness analysis will be presented.
2017 San Antonio Breast Cancer Symposium

Publication Number: P4-02-02

Title: Contrast-enhanced MRI does not accurately predict pathologic complete response (pCR) after neoadjuvant chemotherapy in early or locally advanced breast cancer

Simon P Gampenrieder¹, Andreas Peer¹, Christian Weismann², Matthias Meissnitzer², Gabriel Rinnerthaler¹, Johanna Webhofer¹, Theresa Westphi¹, Marina Popovscaia³, Thomas Meissnitzer², Roland Reitsamer¹, Cornelia Hauser-Kronberger⁵, Heike Egger², Klaus Hergan², Brigitte Mlineritsch¹ and Richard Greil¹. ¹Paracelsus Medical University Salzburg, Salzburg, Austria; ²Paracelsus Medical University Salzburg, Salzburg, Austria; ³Medical University of Innsbruck, Innsbruck, Austria; ⁴Paracelsus Medical University Salzburg, Salzburg, Austria and ⁵Paracelsus Medical University Salzburg, Salzburg, Austria.

Body: Background: Patients with early or locally advanced breast cancer achieving a pathologic complete response (pCR) after neoadjuvant chemotherapy have a lower risk for recurrence or death compared to patients with residual invasive cancer. In the future, breast surgery might be avoided in patients in whom the presence of residual tumor after neoadjuvant therapy can be ruled out with very high confidence. Magnetic resonance imaging (MRI) has been shown to be the most accurate radiologic tool in breast cancer diagnostics and follow-up care. Therefore, we investigated the diagnostic accuracy of contrast-enhanced MRI to predict a pathological complete remission after neoadjuvant chemotherapy.

Methods: This retrospective study included all non-metastatic breast cancer patients treated with neoadjuvant chemotherapy followed by a contrast-enhanced MRI and breast cancer surgery at our institution between 09/2006 and 05/2016. Three specialized breast radiologists, blinded to the clinical and pathological data, reevaluated all preoperative MRI scans and recorded the presence or absence of contrast enhancement as indicator for residual cancer. pCR was defined as no invasive tumor in breast and axilla (ypT0/is N0), however 3 alternative definitions were investigated as well (ypT0 N0, ypT0/is and ypT). Cross tables were used to calculate sensitivity, specificity, pCR-predictive value (PPV), non-pCR-predictive value (NPV) and accuracy. P-values reflecting PPV and NPV differences between various patient subgroups were calculated with Fisher's exact test. The Kaplan-Meier method was used for estimates of distant-recurrence-free survival (DRFS) and overall survival (OS).

Results: In total, 246 patients fulfilled the inclusion criteria and were evaluated. Overall pCR and radiologic complete remission (rCR) rate were 29% and 45%, respectively. Only 48% of rCR corresponded to a pCR (PPV). Conversely, in 87% of cases, residual tumor in the MRI was pathologically confirmed (NPV). The sensitivity to detect a pCR was 75%, while specificity and accuracy were 67% and 69%, respectively. The diagnostic performance of MRI to predict treatment response varied between different histologic and molecular tumor subtypes, however there were little differences between the 4 pCR definitions. The PPV was significantly lower in the hormone-receptor(HR)-positive subgroup compared with the HR-negative subgroup (33% vs. 61%; P = 0.004), especially in luminal-A-like (7%) and lobular carcinomas (0%), respectively. Despite the low concordance (Kohens kappa -0.1), the prognostic value for distant recurrence-free survival was similar between rCR (HR 0.29) and pCR (HR 0.27), respectively. The median pathologic tumor size of residual disease in false-positive cases (rCR but no pCR) was 0.7 cm (SD 0.98 cm).

Conclusion: Contrast-enhanced MRI does not accurately predict pCR after neoadjuvant chemotherapy in early or locally advanced breast cancer, especially in HR-positive tumors. However, in case of false rCR the dimension of residual disease is generally small. Since residual tumor tissue can be detected with high precision, preoperative breast MRI is still of value for operation planning.
2017 San Antonio Breast Cancer Symposium

Publication Number: P4-02-03

Title: Pre-neoadjuvant therapy MRI phenotype can predict response to neoadjuvant endocrine therapy

Talal Hilal¹, Matthew Covington¹, Barbara Pockaj¹, Donald Northfelt¹, Teresa Wu¹, Christine Zwart¹, Jing Li¹ and Bhavika K Patel¹.

¹Mayo Clinic, Phoenix, AZ.

Body: OBJECTIVE: Neoadjuvant endocrine therapy (NET) is increasingly used for the treatment of low and intermediate grade, hormone receptor positive, HER2 negative breast cancer. Several MRI phenotypes that may predict response to neoadjuvant chemotherapy (NAC) have been identified, but little data exists for phenotypes associated with response to NET. This study analyzed imaging phenotypes for all patients treated with NET with the aim to identify specific features that can be predictive of response to therapy.

MATERIALS AND METHODS: The study was retrospective and included 21 patients with clinical stage I, II, and III breast cancer. The tumors were grade 1 or 2, estrogen receptor (ER) positive in >20% of cells, and HER2 non-amplified. MRI examinations were performed in all women before NET. MRI interpretation included mass shape, non-mass enhancement (NME) pattern, background parenchymal enhancement, and MRI phenotype (I well-defined unilocentric mass; II well defined multilobulated mass; III area enhancement with nodularity; IV area enhancement without nodularity; V septal spreading). Type of neoadjuvant endocrine therapy included: tamoxifen alone, an aromatase inhibitor (AI) alone, AI + ovarian suppression, and AI + a non-chemotherapeutic agent. Patients received NET for a total duration ranging between 3 - 6 months, with one patient receiving therapy for 18 months. Clinically meaningful response was defined as stable or decreased tumor size by clinical exam and confirmed at resection by comparing final pathologic T stage with clinical T stage.

RESULTS: Twenty-one patients were identified. Median age was 62 (range 36-84) years. Most were post-menopausal 17 (81%). Pre-neoadjuvant median tumor size on MRI was 3.9 (range 1.0-7.5) cm and comprised T1 3 (14.3%), T2 8 (38.1%), T3/4 10 (47.6%). Pre-treatment N stage was N0 14 (66.7%), N1 7 (33.3%) and pre-NET stage was I in 3 (14.3%), II in 8 (38.1%), and III in 10 (47.6%) patients. The majority 17 (81%) had some tumor reduction, and 4 (19%) had no response. No one achieved a complete response. Of the 17 responders, 7 (41%) had a good response defined as >25% decrease in tumor size. Median tumor size after NET was 3.1 (range 0.6-11) cm and the distribution of T stage was T1 7 (33.3%), T2 9 (42.9%), and T3/4 5 (23.8%). Eleven of 12 (92%) patients with well-defined phenotypes had a response as compared to 6 of 9 (67%) patients with non-well defined phenotypes. Phenotype was not predictive of a good response to therapy, 4 were in the well-defined phenotype and 3 were in the non-well defined phenotype groups. All 4 non-responders had moderate or marked background enhancement as compared to 5 of 17 responders (p = 0.02).

CONCLUSION: A well-defined pre-treatment MRI phenotype was significantly predictive of a positive response to NET, while a non-well-defined MRI phenotype and higher degree of background enhancement was significantly predictive of negative response to NET. This warrants further prospective evaluation, especially in association with Ki-67 levels. If validated, pre-treatment MRI phenotype can be applied in the clinical decision to either initiate NET or referral for upfront surgical resection.
2017 San Antonio Breast Cancer Symposium

Publication Number: P4-02-04

Title: Quantitative MRI features analysis for differentiation of triple negative and HER2 positive subtypes of breast cancer

Gaiane M Rauch1, Heng Li1, Hongtu Zhu1, Beatriz E Adrada1, Lumarie Santiago1, Rosalind P Candelaria1, Hao Wang1, Jessica Leung1, Alastair Thompson1, Jennifer Litton1, Yun Wu1, Bora Lim1, Stacy Moulder1, Elizabeth A Mittendorf1 and Wei Yang1.

1The University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Objective: The aim of this study was to evaluate ability of quantitative analysis of MRI features to distinguish triple negative (TN) and HER2 positive (HER2+) subtypes of breast cancer, which have different biological characteristics, exhibiting different growth patterns and response to treatment.

Materials and Method: Breast cancer patients, who had MRI exam of the breast in our institution at the time of staging for breast carcinoma and who subsequently had surgery (segmentectomy or mastectomy) from January 1, 2008 through December 31, 2015 were identified. All lesions were evaluated by radiologists in accordance with the BI-RADS lexicon. The patient's age, breast cancer histology, multifocality/multicentricity (MF/MC), lesion size, axillary lymphadenopathy (LAN), MRI morphologic and enhancement characteristics were documented. Quantitative MRI feature analysis was performed using shape, texture, and histogram based features. Machine-learning-based (Xgboost) models were used to predict subtypes, and Leave-one-out cross-validation (LOOCV) was used to avoid model overfitting. Statistical significance was determined using the Student's t-test.

Results: Total of 105 patients, 51 patients with TN and 54 patients with HER2+ breast cancer were included in analysis. Mean age for TN was 50 (range 29-79) years old and for HER2+ was 49 (range 25-70) years old. Axillary LAN and MF/MC disease was seen more commonly in HER2+ patients when compared to TN patients, but didn't reach statistical significance (13 vs 7, p=0.9; and 31 vs 20, p=0.06, respectively).

Mass rim enhancement was associated with TN subtype and irregular mass enhancement was associated with HER2+ subtype of breast cancer (p<0.05). Quantitative analysis showed that six out of the top 10 ranked MRI features: surface to volume ratio, difference variance, difference entropy, inverse difference moment, 75 percentile in histogram and sum average, were significantly different between these 2 subtypes with p<0.05. When the significant features were incorporated to distinguish TN and HER+ subtypes, use of the top 2 features achieved the best accuracy on LOOCV of 0.69.

Conclusion: The quantitative MRI features show promise in distinguishing TN and HER2+ breast cancer subtypes reflecting their underlying biological characteristics and may be used as predictive quantitative biological markers. Further studies in a larger cohort evaluating associations with treatment response are underway.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Index</th>
<th>P-value</th>
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</tbody>
</table>
Title: Diagnostic value of breast MRI in evaluating total extent of disease when non-calcified DCIS is present in stereotactic core biopsy samples

Deepa Sheth¹, Jean Bao¹, Hiroyuki Abe¹ and Nora Jaskowiak¹. ¹University of Chicago Medicine, Chicago, IL.

Body:

PURPOSE
To compare the extent of disease as seen on mammography, breast MRI and final surgical pathology in all patients with non-calcified DCIS identified on stereotactic core biopsy. Can MRI provide useful diagnostic information to the surgeon when mammographically occult DCIS is present.

METHOD AND MATERIALS
All patients with stereotactic core biopsies performed at the University of Chicago from 2010 to 2017 were retrospectively reviewed. Patients with pathology demonstrating DCIS in non-calcified cores on stereotactic biopsy were further selected based on whether they had a subsequent breast MRI and definitive surgical treatment. A total of 76 patients met these criteria. All patients undergoing stereotactic core biopsy had at least six 9-gauge cores removed, all of which were radiographed and separated based on whether calcifications were present or absent. Select patients with documented DCIS in the non-calcified cores had a MRI. All patients with MRI underwent a routine diagnostic protocol consisting of one pre- and five post-contrast bilateral, fat suppressed T1 sequences along with a non-fat suppressed T2 sequence. Final surgical treatment was subsequently determined and performed by the breast surgeon.

RESULTS
76 women met all criteria for this study. In 16/76 (21%) of the cases, MRI revealed greater extent of disease than was detected via mammography, and led to change in management in half of those cases (8/76 - 11%). In all 16 of these cases, final surgical pathology confirmed the MRI-detected extent of disease. In 52/76 (68%) of the cases, MRI confirmed the extent of disease detected on mammography and final surgical pathology. In 8/76 (11%) of the cases, MRI showed little to no abnormal enhancement at site of mammographically detected calcifications, even though final surgical pathology confirmed presence of residual disease.

CONCLUSION
The presence of non-calcified DCIS in stereotactic core biopsy samples raises the suspicion for the presence of mammographically occult disease. Breast MRI performed in this setting shows that in majority (82% -68/76) of the cases, MRI can either confirm or even advantageously reveal greater extent of disease than was initially detected on mammography. Furthermore, there can be a change in management due to the breast MRI findings.

CLINICAL RELEVANCE/APPLICATION
Breast MRI can be a useful diagnostic tool for the surgeon when non-calcified DCIS is present in stereotactic core biopsy samples.
Title: Intratumoral and peritumoral MRI signatures of HER2-enriched subtype also predict pathological response to neoadjuvant chemotherapy in HER2+ breast cancers

Nathaniel Braman¹, Prateek Prasanna¹, Salendra Singh², Niha Beig¹, Hannah Gilmore², Maryam Etesami², David Bates¹, Katie Gallagher¹, B Nicolas Bloch¹, George Somlo⁵, William Sikov⁶, Lyndsay Harris³, Donna Plecha², Vinay Varadan² and Anant Madabhushi¹. ¹Case Western Reserve University, Cleveland, OH; ²Case Comprehensive Cancer Center, Cleveland, OH; ³National Institutes of Health; ⁴Boston Medical Center, Boston, MA; ⁵City of Hope Beckman Research Institute and Medical Center, Duarte, CA and ⁶Brown University, Providence, RI.

Body: Background: Applying the PAM50 classifier to targeted RNA-Sequencing data allows HER2+ tumors to be sub-categorized into intrinsic breast cancer subtypes. HER2+ breast cancers belonging to the HER2-enriched [HER2-E] subtype exhibit the highest rate of response to neoadjuvant therapy with combination of HER2-blockade and chemotherapy, as well as dual-HER2 blockade alone. A non-invasive predictor of PAM50 subtype from clinical dynamic contrast-enhanced MRI [DCE-MRI] could provide valuable clinical guidance in the treatment of HER2+ breast cancer. In this work, we identify a set of computer-extracted heterogeneity features computed within the lesion and its surrounding peritumoral region capable of distinguishing HER2-E from other HER2+ breast cancers [Non-HER2-E]. We then demonstrate that this imaging signature of HER2-E is also predictive of pathological complete response [pCR] in an independent HER2+ testing set, consistent with the HER2-E subtype’s elevated response to HER2-targeted therapy.

Methods: The training set consisted of 42 HER2+ patients with both 1.5 or 3 T DCE-MRI and targeted RNA sequencing collected prior to neoadjuvant treatment from a multicenter trial [BrUOG 211B, n=35] and The Cancer Genome Atlas-Breast Cancer project [TCGA-BRCA, n=7]. Intrinsic subtypes were assigned by unsupervised hierarchical clustering of the PAM50 gene set. 19 patients were determined to belong to the HER2-E subtype, while the remaining 23 represented non-HER2-E subtypes [19 HER2-Luminal, 4 HER2-basal]. Lesion boundaries were annotated by an expertly trained radiologist and expanded to 5 annular peritumoral regions in 3 mm increments out to a maximum radius of 15 mm. Computer-extracted heterogeneity features were computed voxelwise within intratumoral and peritumoral regions by first order statistics. A top HER2-E-associated feature from each region was identified by Wilcoxon feature selection and used to train a diagonal linear discriminant analysis [DLDA] classifier to predict HER2-E in a 3-fold cross-validation setting. This classifier was then applied to pCR prediction from DCE-MRI in a testing set of 28 HER2+ patients with available post neoadjuvant chemotherapy surgical specimens at one institution. 16 patients achieved pCR (ypT0/is), while the remainder had partial or no response (non-pCR).

Results: A combination of heterogeneity features within the intratumoral region and annular peritumoral regions out to 12 mm from the tumor yielded optimal results within the training set, with an average HER2-E prediction AUC of .77 +/- .03. When applied to response prediction in an independent testing set, this HER2-E classifier was predictive of pCR (AUC = .72).

Conclusions: Computer-extracted heterogeneity features calculated within the tumor and the surrounding peritumoral environment on DCE-MRI were able to distinguish the HER2-E PAM50 intrinsic subtype from other HER2+ breast cancers. HER2-E was characterized by elevated expression of intratumoral and peritumoral heterogeneity features, indicating a more disordered imaging phenotype within and around the tumor. Additional independent validation of these findings is needed.
Title: Radiogenomic analysis of HER2+ breast cancer reveals MRI features correlated with genomic immune index are predictive of neoadjuvant chemotherapy response

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Body: Background: It has been previously shown that computer-extracted heterogeneity features calculated within the peritumoral region of a breast cancer from baseline dynamic contrast-enhanced MRI [DCE-MRI] can predict treatment outcome. This approach may due to microenvironmental signatures of elevated immune response, a predictor of favorable response to neoadjuvant chemotherapy [NAC] in HER2+ breast cancer. We assessed the correlation between peritumoral radiomic features and a tissue-derived genomic immune index [II] in HER2+ breast cancer and whether these immune-correlated imaging features are predictive of pathologic response.

Methods: 33 HER2+ patients with both 1.5 or 3 T DCE-MRI imaging and targeted RNA sequencing of biopsy samples collected prior to NAC from a multicenter trial [BrUOG 211B, n=26] and The Cancer Genome Atlas-Breast Cancer project [TCGA-BRCA, n=7] were retrospectively analyzed. II was derived from expression of a 140-gene immune signature using the ESTIMATE algorithm. An attending breast radiologist annotated lesion boundaries on the DCE-MRI phase of peak contrast enhancement. Beyond this intratumoral region, 5 annular peritumoral regions in 3 mm increments out to a maximum radius of 15 mm were analyzed. Computer-extracted heterogeneity descriptors computed within the intratumoral and peritumoral regions were summarized by first order statistics. Redundancy was reduced by eliminating correlated imaging features (R^2>.6). From the remaining features, the 5 features that were collectively best correlated with II were selected by feed forward, leave-one-out multilinear regression. The regression model was applied to an independent test set of 28 HER2+ patients with post NAC surgical specimens. The estimated II was assessed for its ability to differentiate patients who achieved a pathologic complete response in the breast [pCR, ypT0/is] (n=16) and those who did not (n=12) by 2-sided Wilcoxon rank sum test of median and area under the receiver operating characteristic curve (AUC).

Results: The set of top features that significantly correlated (p<.05) with II were entirely within the 3-6 mm and 6-9 mm peritumoral regions. These features characterized uneven patterns of enhancement and heterogeneity of intensity values. Within the testing set, predicted II was significantly higher among responders (pCR: 12.8 +/- .3, non-pCR: 12.5 +/- .3, p<.05) and distinguished the two with an AUC of .73.

Conclusions: From a set of quantitative features characterizing heterogeneity within the peritumoral region on DCE-MRI, we identified peritumoral imaging features correlated with a genomic index of immune response in HER2+ breast cancer and were predictive of pathologic response in an independent testing set. Our findings suggest that the predictive capability of peritumoral radiomics may be tied to a patient's immune response to the cancer. In addition to providing insight to the biological basis of peritumoral radiomics, imaging signatures of immune response themselves possess clinical value as a potential means for the non-invasive prediction of HER2+ cancer biology and treatment outcome. Additional independent validation is needed on a larger test set to confirm our preliminary findings.
2017 San Antonio Breast Cancer Symposium

Publication Number: P4-02-08

Title: Repeatability and reproducibility of quantitative breast MRI in community imaging centers: Preliminary results

Anna G Sorace¹, Jack Virostko¹, Chengyue Wu¹, Angela M Jarrett¹, Stephanie L Barnes¹, Jeffrey Luci¹, Debra A Patt², Boone Goodgame¹, Sarah Avery³ and Thomas E Yankeelov¹. ¹University of Texas at Austin, Austin, TX; ²Texas Oncology, Austin, TX and ³Austin Radiological Association, Austin, TX.

Body: Introduction: The primary purpose of this study is to evaluate the repeatability and reproducibility of quantitative breast MRI across community imaging centers with the ultimate goal of using these techniques to predict breast cancer response early in the course of neoadjuvant therapy (NAT). Dynamic contrast-enhanced MRI (DCE-MRI), diffusion-weighted MRI (DW-MRI), and magnetization transfer MRI (MT-MRI) performed early in the course of breast NAT has the potential to predict eventual response prior to changes in tumor size. This enables tailoring of treatment plans and the opportunity to substitute ineffective therapies with alternative approaches. We present preliminary results on the reproducibility and repeatability of $T_1$, apparent diffusion coefficient (ADC), and magnetization transfer ratio (MTR) measurements in normal breast fibroglandular tissue (FGT) in the community setting.

Experimental Design: MRI was performed at two community imaging centers and one academic research facility using 3T Siemens Skyra scanners equipped with 8- or 16-channel breast coils. To assess repeatability of the imaging techniques, normal subjects (N=10, ages 22-62) were scanned twice, separated by subject repositioning. To assess reproducibility across sites, normal subjects (N=3) were scanned at three imaging centers. To assess quantitative $T_1$ measurements, subjects were scanned using a spoiled gradient echo (SPGE) sequence and variable flip angles (2, 4, 6, ..., 20) with TR/TE = 7.9/2.71 ms and corrected for $B_1$ inhomogeneity. To assess the ADC, subjects were scanned using an echo-planar monopolar spin echo sequence with the following parameters: TR/TE = 3000/52 ms and $b$-values = 0, 200, 800 s/mm². MT-MRI was acquired using two gradient echo sequences with TR/TE = 48.0/6.40 ms, one with the inclusion of a 1500 Hz off-resonance saturation pulse. FGT was segmented using k-means clustering. Women undergoing NAT for breast cancer are being recruited and scanned with DCE-MRI, DW-MRI, and MT-MRI at baseline (prior to beginning therapy) and three early time points during the course of NAT to evaluate early prediction of response to therapy.

Results: Reproducibility scans of normal breast FGT yielded an average difference of 8.4% in $T_1$ measurement, 7.0% in ADC measurement, and 12.7% in MTR measurement between sites. Repeatability scans of the same subject’s FGT showed an average percent difference of 6.7% in $T_1$ measurement, 4.5% in ADC measurement, and 11.6% in MTR measurement between the two scans. A multi-site trial performing quantitative DCE-MRI, DW-MRI, and MT-MRI in patients undergoing breast NAT in the community setting (N=16 at the time of submission) has been ongoing to predict response to NAT using quantitative MRI.

Conclusion: Quantitative DCE-, DW-, and MT-MRI of the breast is both repeatable and reproducible across MRI scanners in community imaging centers. A quantitative breast MRI protocol can be deployed at community imaging centers for breast cancer patients. These results and ongoing work highlight the feasibility of future clinical dissemination of quantitative MRI for predicting early response to NAT, therefore expanding these novel techniques to a widespread patient population.

We acknowledge the support of CPRIT RR160005.
Body: Objective: To assess the association of MRI BPE and pathological response in women diagnosed with stage II/III breast cancer submitted to NAC. Methods: This observational and cross-sectional retrospective study was performed in consecutive women who underwent NAC and had MRI exams before and after chemotherapy. The MRI was done before and after 2 weeks of completing NAC. BPE was classified according to ACR-BIRADS 5th edition. The type of BPE before NAC, its changes and the relationship to total pathologic complete response (TpCR) were evaluated. Data were paired with patient age, size on MRI before and after NAC, features of clinical response according to the RECIST criteria, tumor grade and immunohistochemical (IHC) subtypes. MRI assessment included amount of fibroglandular tissue, symmetry of BPE and measurement of tumor at the longest diameter. All images were blinded reviewed by a radiologist. We used for the changes of the BPE the Bowker symmetry test or the McNemar test and to analyze the factors related to the clinical and pathologic responses, logistic regression analysis. The level of significance adopted was 5% (p<0.05). Results: We studied 71 women between 2009 and 2016. The medium age was 37 years old. BPE was symmetrical in 68 women (95.8%). Moderate and marked BPE was present in 28 (39.4%) of the affected breasts and in 25 (34.2%) of the contralateral breasts. After NAC all BPE were symmetrical and just 3% of them were moderate or marked. Regarding the IHC subtype, 40 women (56.3%) were triple negative or HER2 positive, and these women had a higher frequency of TpCR (55% for each, compared to 12.9% in patients with luminal subtypes). We found to be independently associated with pCR: the reduction of BPE (in the affected or contralateral breast) and the molecular subtypes triple negative and HER2 positive.

Table 1. Multivariate Analysis related to TpCR (n=71).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>P-Value</th>
<th>O.R.*</th>
<th>CI 95% O.R.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor Size on MR pre-MAC (cm)</td>
<td>Luminal B (ref.)</td>
<td>0.171</td>
<td>0.859</td>
<td>0.691-1.068</td>
</tr>
<tr>
<td>Luminal subtype</td>
<td>Luminal A</td>
<td>0.312</td>
<td>0.45</td>
<td>0.10-2.11</td>
</tr>
<tr>
<td>HER2pos/ Luminal B HER2</td>
<td>0.005</td>
<td></td>
<td>5.78</td>
<td>1.71-19.58</td>
</tr>
<tr>
<td>Triple negative</td>
<td>0.049</td>
<td></td>
<td>3.27</td>
<td>1.01-10.64</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td>0.387</td>
<td>0.982</td>
<td>0.942-1.023</td>
</tr>
<tr>
<td>Nottingham grade</td>
<td>1 (ref.)</td>
<td>---</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.081</td>
<td>7.83</td>
<td>0.78-79.16</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.096</td>
<td>7.10</td>
<td>0.71-71.31</td>
</tr>
<tr>
<td>BPE pre-NAC (S or A)</td>
<td>Asymmetric (ref.)</td>
<td>---</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Symmetric</td>
<td>0.327</td>
<td>3.03</td>
<td>0.33-27.76</td>
</tr>
<tr>
<td>BPE pre-NAC affected breast</td>
<td>Minimal (ref.)</td>
<td>---</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mild</td>
<td>0.812</td>
<td>0.86</td>
<td>0.24-3.09</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>0.371</td>
<td>1.89</td>
<td>0.47-7.64</td>
</tr>
<tr>
<td></td>
<td>Marked</td>
<td>0.591</td>
<td>1.57</td>
<td>0.30-8.17</td>
</tr>
<tr>
<td>BPE pre-NAC contralateral breast</td>
<td>Minimal (ref.)</td>
<td>---</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mild</td>
<td>0.713</td>
<td>0.79</td>
<td>0.22-2.81</td>
</tr>
<tr>
<td>BPE Change</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>-----</td>
<td>-----</td>
<td>---------</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>0.250</td>
<td>2.33</td>
<td>0.55-9.77</td>
<td></td>
</tr>
<tr>
<td>Marked</td>
<td>0.470</td>
<td>1.88</td>
<td>0.34-10.43</td>
<td></td>
</tr>
<tr>
<td>Same/increased (ref.)</td>
<td>---</td>
<td>1.00</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Reduction</td>
<td><strong>0.026</strong></td>
<td>3.01</td>
<td>1.14-7.96</td>
<td></td>
</tr>
</tbody>
</table>

* OR (Odds Ratio) = Risk ratio to pCR; (n=26 pCR, n=7 DpCR, n=31 PR e n=7 ED, where Total pCR is pathological complete response (without invasive and DCIS in the breast and axilla) DpCR is pathological response with just DCIS, PR is partial response and ED is stable disease – we haven't progression disease). CI 95% OR = Confidence interval of 95% to risk ratio. Ref.: reference level. Proportional risk models.

**Conclusion:** BPE reduction was significantly associated with TpCR. Nevertheless, patterns of BPE pre-NAC have no association with pathological response.
Title: Breast cancer surveillance in high-risk women with dynamic contrast-enhanced magnetic resonance imaging every 6 months: Results from a single institution study

Kristen Whitaker1, Rodrigo Guindalini, Hiroyuki Abe1, Deepa Sheeth1, Dezheng Huo1, Susan Hong1, Jane Churpek1, Marion Verp1, Elias Obeid1, Yonglan Zheng1, Andrea Amico1, Toshio Yoshimatsu1 and Olufunmilayo Olopade1. 1The University of Chicago Medicine, Chicago, IL.

Body: Purpose: To develop a novel approach for early detection of breast cancer and examine molecular features of screen detected cancers in prospectively ascertained high-risk women undergoing semi-annual dynamic contrast-enhanced breast magnetic resonance imaging (DCE-MRI) for women at high genetic risk.

Background: Women with a personal or family history of breast cancer and genetic mutation carriers of BRCA1 and BRCA2 have a higher than normal risk of breast cancer. An intensified screening surveillance regimen is an early detection strategy in high-risk women. The American Cancer Society recommends annual DCE-MRI in addition to annual mammogram based off several pivotal screening studies that demonstrated improved sensitivity and cancer detection rates and decreased interval cancer rates with the addition of annual DCE-MRI. Questions remain regarding the optimal screening modality and interval regimen in these high-risk women.

Methods: Between 2004 and 2016, we assembled a prospective cohort of high-risk women undergoing semi-annual DCE-MRI and annual mammography. To be eligible, women had a lifetime breast cancer risk >20% and/or tested positive for a pathogenic mutation using a cancer gene panel including BRCA1, BRCA2, CDH1, PALB2, CHEK2 and other cancer susceptibility genes in the DNA repair pathway. Somatic mutation events in screen-detected tumors were investigated using UW-OncoPlex cancer gene panel using DNA extracted from FFPE shavings.

Results: 295 women were recruited to the study; 44% of the study participants had pathogenic mutations in BRCA1 or BRCA2 genes. At a median follow-up of 3.3 years (range 0-12 years), 3 DCIS and 13 early stage invasive breast cancers were detected, of which 14 occurred in subjects with identifiable pathogenic mutations (11 BRCA1, 2 BRCA2, 1 CDH1). The incidence rate is 1.3% in all subjects, but 3.5 % per year in BRCA1 carriers. DCE-MRI identified all 13 invasive cancers at a mean size of 0.61 cm (range 0.1-1.0 cm); none had lymph node metastasis. No interval cancers occurred. In addition, 7 of the breast cancers were detected on DCE-MRI imaging obtained at the 6 months screening interval; they would be interval cancers if only annual screening were implemented. There was very little DNA for somatic mutation testing in the majority of cases. However, as expected, there was heterogeneity in the spectrum of mutations but the most commonly somatically mutated gene in the early cancers was TP53.

Conclusions: DCE-MRI every 6 months performed well for early detection of invasive breast cancer in high-risk women, accomplishing the ultimate goal of breast cancer screening—detecting node-negative, invasive tumors less than 1 cm. Semi-annual DCE-MRI performed especially well in BRCA1 mutation carriers at risk for the most aggressive subtype of breast cancer. Further interventional studies evaluating this novel screening approach are warranted to personalize breast cancer risk assessment and prevention.
2017 San Antonio Breast Cancer Symposium

Publication Number: P4-02-11

Title: Accuracy of breast magnetic resonance imaging has limited value to reduce the margin-positive rate: A study in relation to the molecular subtypes

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Body: Background: Newly released guideline standardizing a negative margin after breast-conservative surgery (BCS) as “no ink on tumor” by SSO-ASTRO stressed the importance of estimation tumor extent with comprehensive breast imaging studies. To evaluate clinical value of breast magnetic resonance imaging (MRI) in patients with BCS, we compared the degree of correlation between MRI-pathology and ultrasonography (US)-pathology according to subtypes. In addition, we investigated the margin-positive rates and secondary operation rates for margin clearance.

Methods: We identified patients with invasive breast cancer who had preoperative breast MRI and ultrasound between 2011 and 2016. We excluded patients having large tumor more than 5cm or multiple tumors or undergoing mastectomy. Patients were classified into 4 subtypes based on the immunohistochemistry; luminal A, luminal B/HER2, HER2, triple-negative breast cancer (TNBC). Lin’s concordance correlation coefficient was used to measure the agreement between the MRI or US and tumor extent. Tumor extent was defined as pathologic tumor size including in situ carcinoma. Margin-positivity was assessed based on intraoperative frozen examination.

Results: A total 516 patients with single tumor undergoing BCS were included. Means of tumor size were 1.99 ± 0.91 cm by pathologic examination, 1.91 ± 1.01 cm by MRI, and 1.76 ± 0.92 cm by US, respectively. The correlation coefficient of MRI-pathology was significantly higher than that of US-pathology (r=0.6975 vs. 0.6211, P=0.001). A superiority of MRI than US in measuring pathologic extent was only observed in TNBC (r=0.8089 vs. 0.6014, P<0.001), whereas the agreement between the MRI or US and tumor extents was low in the HER2 (MRI: 0.3509, US: 0.3165). Also, the margin-positive rate was higher in HER2 (luminal A, 11.6%; luminal B/HER2, 17.5%; HER2, 29.6%; TNBC, 17.8%; P=0.0382). In the post-hoc test, the HER2 was more likely to have positive margin compared to Luminal A (P=0.0039). There is no significant difference in secondary operation as margin clearance according to the subtypes (P>0.999).

Margin positive and re-excision rates according to the subtypes

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Luminal A (n=302)</th>
<th>Luminal B (n=80)</th>
<th>HER2 (n=27)</th>
<th>TNBC (n=107)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive margin</td>
<td>35 (11.6)</td>
<td>14 (17.5)</td>
<td>8 (29.6)</td>
<td>19 (17.8)</td>
<td>0.0382</td>
</tr>
<tr>
<td>Re-excision</td>
<td>14 (4.6)</td>
<td>4 (5.0)</td>
<td>1 (3.7)</td>
<td>5 (4.7)</td>
<td>&gt;0.9999</td>
</tr>
</tbody>
</table>

Conclusions: Given a superiority of MRI to US in preoperative assessment, MRI-guided BCS did not reduce the margin-positive rate in TNBC. In the HER2, size correlation of MRI-pathology was very low, and the margin-positive rate was high. Collectively, our findings suggest that accuracy of MRI has limited value to reduce the margin-positive rate.
Title: Predicting surgical upgrade of breast lesions detected by MRI-guided biopsy

Angela Chen¹ and Rachel Brem¹. ¹George Washington University, Washington DC.

Body: Background:
Patients who have suspicious breast lesions identified with MRI without mammographic- or ultrasound-correlated biopsy require MRI-guided biopsy. However, MRI-guided biopsy may underestimate the pathology due to intrinsic heterogeneity, in which subsequent surgical excision may reveal an upgrade. The reported upgrade rate is variable across studies, subclasses of lesion, and needle gauge, and no associative factors have been identified. This study investigated demographic and histologic characteristics of lesions that underwent MRI-guided biopsy to determine possible factors for predicting later upgrade at surgery.

Methods:
All MRI-guided vacuum biopsies performed between 2012 and 2017 at GWU Breast Imaging Center were reviewed. Surgical excision was performed for high risk lesions and imaging discordance in benign lesions. Variables investigated include patient age, menopausal status, personal/family history of breast cancer and breast pathology. T-test and Pearson's chi-squared test were used to determine statistical significance (alpha=0.05).

Results:
Of 379 lesions from 295 patients that underwent MRI-guided biopsy, 117 lesions from 96 patients underwent subsequent surgical excision. Pathology of the 117 lesions included benign (14/117; 12%), ALH (17/117; 15%), LCIS (10/117; 9%), ADH (10/117; 9%), papilloma (25/117; 21%), radial scar (12/117; 10%), DCIS (13/117; 11%), invasive cancer (10/117; 9%), and others (6/117; 5%). 14 of 117 lesions (12%) were upgraded at surgery: 5 upgraded from benign to high-risk, 1 upgraded from benign to DCIS, 4 upgraded from high risk to DCIS, 1 upgraded from high risk to invasive cancer and 3 upgraded from DCIS to invasive cancer. All 14 patient with upgrades (100%) had a personal history of breast cancer. No association was found with age, menopausal status, breast pathology, or family history.

Conclusions:
Our study demonstrated a surgical upgrade rate of 12%. All upgrades occurred in women with a personal history. We suggest that patients with a personal history of breast cancer pose a higher risk of surgical upgrade at excision.
**Title:** Can background parenchymal enhancement (BPE) predict the chance of multicentric/bilateral breast cancer on staging breast MRI?

Ana Paula A Benveniste¹, Lilian O Ebuoma¹, Arti Jonna¹, Kenny Sam¹, Frederich J Severs Jr.¹, Ashley Roark¹, Karla A Sepulveda¹, Ortiz-Perez Tamara¹ and Emily E Sedgwick¹. ¹Baylor College of Medicine, Houston, TX.

**Body:** Purpose: Can background parenchymal enhancement (BPE) predict the chance of multicentric/bilateral breast cancer on staging Breast MRI?

Materials and Methods: Retrospective review of women diagnosed with breast cancer who underwent a breast MRI for staging of disease was performed at a single institution from October 2012 to September 2015.

Results: Breast MRI for staging was done for 435 patients diagnosed with breast cancer. Ages ranging from 24 to 86 years old (median, 52 years); sizes ranging from 0.5 to 13.5 cm (mean, 3.5 cm). 29 patients (7%) had biopsy-proven second tumors in the same breast but different quadrant as the primary tumor or in the contralateral breast. From the 435 patients, 304 had minimal/mild BPE and 103 had moderate/marked BPE (28 missing). Of the patients with an invasive primary cancer and a second tumor in the same breast but different quadrant as the primary tumor or in the contralateral breast, 12 of 29 patients had discordance in subtypes between the primary and secondary tumors (41%) and 17 of 29 had concordance in the subtypes between the primary and secondary tumors (59%). 6.3% (19 out 304) of patients with minimal/mild BPE had multicentric/bilateral disease and 9.7% (10 out 103) of patients with moderate/marked BPE had multicentric/bilateral breast cancer.

Conclusion: 435 patients with recent diagnosis of breast cancer who underwent staging breast MRI for diagnosis of additional sites of disease, 7% was diagnosed with an additional site of cancer. From these group of patients, approximately 6% had other site of disease among patients who had minimal/mild BPE in the breast MRI versus 10% of patients with moderate/marked BPE. BPE may play a role in the setting of multicentric/bilateral breast cancer.
Title: Causes of endocrine therapy resistance: An in-depth genomic analysis of resistant multidrug ER+ breast cancers

J Michael Dixon², Arran K Turnbull³, Maki Tanioka³, Amy Wheless³, Amy Garrett³, Carlos Martinez-Perez¹, Joel Parker³, Xiaping He³, Andrew H Sims¹, Jeremy S Thomas⁴, Lisa A Carey³ and Charles M Perou³. ¹Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, United Kingdom; ²Edinburgh Breast Unit, Western General Hospital, Edinburgh, United Kingdom; ³Lineberger Comprehensive Cancer Cente, University of North Carolina, Chapel Hill, NC and ⁴Western General Hospital, Edinburgh, United Kingdom.

Body: Background: 70% of all breast cancers (BCs) are ER+. Not all ER+ cancers respond to endocrine therapy (ET) and many eventually develop resistance. The aim was to perform in-depth genomic analysis of both primary resistant BCs, that do not respond to ET, and cancers which progress (>40% increase in size) after an initial response as they acquire resistance (AQR) to ET.

Methods: A unique series of 48 post-menopausal women with ER+ BC received neoadjuvant ET using letrozole (L) or anastrozole (A) (mean treatment duration 17 months, range 3-67). 13/48 received up to 4 lines of ET. 12/48 responded to A or L, 16/48 had primary resistance and 20/48 had AQR.

Of 20 with AQR, 13 had 2nd line ET with A or tamoxifen (T). 6 had 3rd line ET with exemestane (E) and 1 had 4th line megestrol acetate (MA). Serial RNA & DNA from 3-5 cancer samples/patient (226 samples) had Ribo0-RNAseq, DNA exome sequencing and somatic mutation detection using UNCeqR. We have data so far on 29 patients: 5 responders, 4 with primary resistance and 20 AQR, the full cohort will be complete shortly.

Results:
ESR1 Mutations (ESRM): 1/5 responders had an ESRM (E380Q) at diagnosis. This clone disappeared with response to L. 5/20 patients with AQR (25%) had clonal expansion of an ESRM during 1st line ET (L:4, A:1). 4 had a chr6:152419926[lowbar]A:G (D538G) ESRM and 1 had a novel ESRM. Of the 5 with ESRM acquired during 1st line ET, the mutant allele fraction (MAF) increased further in the 4 who had 2nd ET (3:T, 1:E) and increased further for the 2 who had 3rd line E.

ESR1 Amplification (ESRA): 5 patients developed ESRA. 3/5 developed ESRA on 2nd or 3rd line E that was not present on AQR to 1st line L or A and 2nd line T. The other 2 developed ESRA on L. 2/5 with ESRA had concomitant CYP19A1 amplification. One patient with ESRA that developed on 3rd line E subsequently responded to MA. No patients with primary resistance to 1st line ET had an ESRM or ESRA.

PIK3CA mutations (PIK3M): 5/20 with AQR had PIK3M (25%). 3/7 had PIK3M at diagnosis and in 3 MAF increased between 1st and 2nd line ET. 2/7 developed PIK3M when resistant to 2nd line ET, 1 of the 2 had ESRA. 2 patients responsive to L had PIK3M at diagnosis and MAF decreased with therapy.

Other Mutations: Unique mutations with limited commonality developed and new clones expanded in the remaining cancers during primary and acquired resistance. Clonality analysis of AQR samples to different ETs showed proliferation of specific clones, characterised by novel sets of mutations, which typically became the dominant clone at the time of resistance to a particular agent.

Summary: 13/20 with acquired resistance had ESRM, ESRA, or PIK3M in resistant tumours: 1 had all 3, 2 ESRM + ESRA, 1 ERSA + PIK3CA, 4 ESRM only, 2 ESRA only and 3 had PIK3M only.

Conclusions:
- Endocrine resistance is complex
- ESRM or ESRA is uncommon at diagnosis and does not explain primary ET resistance
- ESRM (in particular the D538G mutation) occurs in one-third of patients with acquired resistance. 2nd line ET results in clonal selection and expansion of ESRM cells. Assessing recurrences for ESRM by in situ detection has clinical utility
- ESRA is only seen in heavily ET-pre-treated tumours, with its significance being unknown.
Title: Characterizing and targeting chemoresistant subclones in patient-derived xenograft models of triple negative breast cancer

Gloria V Echeverria¹, Sahil Seth², Zhongqi Ge¹, Yuting Sun², Emilia DiFrancesco², Rosanna Lau⁴, Joe Marszalek², Stacy Moulder³, Fraser Symmans¹, Timothy P Heffernan², Jeffrey T Chang⁵ and Helen Piwnica-Worms¹. ¹The University of Texas M.D. Anderson Cancer Center, Houston, TX; ²Institute for Applied Cancer Science, The University of Texas M.D. Anderson Cancer Center, Houston, TX; ³The University of Texas M.D. Anderson Cancer Center, Houston, TX; ⁴The University of Texas M.D. Anderson Cancer Center, Houston, TX and ⁵The University of Texas Health Science Center, Houston, TX.

Body: Fifty percent of all triple negative breast cancer (TNBC) patients harbor significant residual tumor burden following treatment with standard neoadjuvant chemotherapy (NACT), resulting in poor prognosis. Recent studies in TNBC have revealed extensive intra-tumoral heterogeneity at the time of diagnosis and throughout disease progression, but the relative contributions of these heterogeneous populations of tumor cells to chemoresistance are not well understood.

The primary tumor, dermal metastasis, and germline reference were obtained from a patient with untreated metastatic TNBC. Tumor cells were engrafted into the humanized mammary fat pads of NOD/SCID mice to establish PDX models of the primary (PIM001-P) and metastatic (PIM001-M) tumors. RNA sequencing and whole-exome sequencing (WES), performed on the patient's primary and metastatic tumors and the first- and third- passage PDX models revealed transcriptomic profiles and subclonal heterogeneity of the patient's tumors were recapitulated in the PDX models.

Treatment of mice engrafted with PIM001-P tumors with NACT (Adriamycin plus cyclophosphamide, AC) resulted in partial response, the magnitude of which was diminished in mice bearing PIM001-M tumors. Tumor subclones were tracked during chemotherapy treatment in mice engrafted with PIM001-P tumors using lentiviral non-targeting DNA barcodes. Residual tumors maintained the clonal architecture of untreated tumors, and deep WES revealed stable maintenance of somatic mutant allele frequencies throughout treatment. Therefore, selection of pre-existing resistant clones did not lead to AC resistance in this model. Interestingly, only 25% of residual tumor clones contributed to primary relapse once treatment was halted, suggesting only a subpopulation of tumor cells was able to reconstitute the tumor.

RNA sequencing and reverse phase protein array revealed that while vehicle-treated and regrown tumors were highly similar, residual tumors harbored a unique profile characterized by numerous significant alterations in RNA and protein levels. Together, these results suggest that residual tumors enter into a transient drug-resistant state that is reversible. Residual tumors were enriched for alterations in pathways such as metabolism, extracellular matrix remodeling, and cell-cell communication.

Pharmacologic targeting of the residual tumor state with an inhibitor of mitochondrial oxidative phosphorylation led to significant inhibition of tumor regrowth following AC treatment. Additional vulnerabilities identified in residual tumors are being targeted therapeutically with the goal of eradicating residual tumor cells.
Title: Breast tumors escape endocrine therapy by the coordinated activities of HER2/3 and FOXA1

Antoni Hurtado1, Shxiong Wang1, Siv Gilfillan1, Jens Henrik Norum1, Helga Bergholtz1, Sachin Kumar Singh1, Anne Marthe Fosdahl1, Silje Nord1, Olav Engebraten1, Ole Christian Lingjaerde1, Meritxell Bellet2 and Therese Sørlie1. 1University of Oslo, Oslo, Norway and 2Vall d’Hebron Institute of Oncology, Barcelona, Spain.

Body: Hormone therapy reduces tumor growth of Estrogen Receptor (ER) positive breast cancer patients. However, relapse to these therapies occurs in a substantial number of patients. Hormone therapy is sequentially used in the treatment of resistant patients. Nevertheless, the results of these therapies are globally poor, with median Progression Free Survival of only 2.8 to 4.5 months (Turner, N Engl J Med 2015). The choice of these treatments is based on the premise that ER is still the main driving force of proliferation in these tumors. We have now analyzed the expression of HER2 in low-grade HER2 negative tumors from patients with who initially responded to hormone therapy but eventually relapsed and compared those to metastases from the same patients once have relapsed. Our results reveal that metastases have increased HER2 expression compared to primary tumor biopsies of the same patient, which suggested the cross talk between HER2 and ER might be the driving force of tumor growth for metastases. Importantly, the signaling of HER2 is complex and its activation is usually dependent of dimerization with other receptor tyrosine kinases in cell membrane such as EGFR or HER3. Our study reveals that EGFR or HER3 use different mechanisms to control the function of ER and the transcription factor FOXA1. Our data confirms that ER and FOXA1 mediate the EGFR signaling in hormone resistant tumors as previously described (Shou, JNCI 2004). By contrast, HER3 confers insensitivity to anti-ER drugs by driving proliferation in an ER independent manner but still dependent of FOXA1. Now, we prove that in response to HER2/3 signaling, FOXA1 controls the transcription of genes associated with poor prognosis and metastases by binding to chromatin regions poorly enriched of ER binding. By doing so, FOXA1 leads to an ER-independent growth and therefore confers insensitive to anti-ER drugs. The role of FOXA1 contribution to breast cancer progression independently of ER is unforeseen, since we previously reported that the ER chromatin interactions are mediated by FOXA1 (Hurtado, Nat Genetics 2011). Now, our study reveals that FOXA1 is acetylated by the acetyltransferase EP300 and that such post-translational modification retains FOXA1 at ER chromatin regions. By contrast, HER2/3 signaling hinders FOXA1 acetylation and facilitates the binding of FOXA1 to chromatin regions poorly enriched of non-ER sites. Moreover, the expression of FOXA1 mutant defective for acetylation mimics the HER2/3 effect and also confers insensitivity to hormone therapy. Altogether, our study supports that ER/EP300 limits the binding of FOXA1 and the ER independent function. By contrast, in hormone resistant tumors with enhanced HER2/3 signaling, FOXA1 becomes less acetylated and now can binds without restrictions to chromatin regions poorly enriched of ER sites. As a consequence, the tumor gains the ability to grow independently of ER and therefore becomes insensitive to anti-ER therapies. Our study provide insight into tumor heterogeneity, revealing mechanistic insight into what tumors should be targeted with specific HER-targeted therapies and defining whether ER is still functional and still valid as a single drug target.
Targeting the mevalonate pathway in HER2-positive breast cancer to overcome resistance to anti-HER2 therapy

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Body: Background: Despite the advent of HER2-targeted therapies for HER2+ breast cancer (BC), including the monoclonal antibody trastuzumab (T) either alone or in combinations, resistance still poses a major clinical challenge. Using our broad panel of HER2+ cell lines made resistant (R) to T alone (TR), and to lapatinib plus T (LTR), we observed that in resistant models where HER2 remains inhibited, especially the LTR derivative, the mevalonate (MVA) pathway is activated and provides an alternative proliferative signal, including the activation of mTOR, to drive resistance. While these resistant cell models were hypersensitive to the widely-used cholesterol-lowering statins, the role of other MVA pathway inhibitors such as zoledronic acid (ZA), which is in clinical use to treat bone metastasis, in overcoming resistance to HER2-targeted therapy has not been explored. Based on recent reports and our preliminary data using reverse phase protein array (RPPA) analysis, the YAP/TAZ transcription factor (TF) emerged as a potential mediator of MVA pathway signaling to mTOR. Here, we investigated the therapeutic efficacy of additional MVA pathway inhibitors and the role of YAP/TAZ in mediating resistance to HER2-targeted therapy.

Methods: HER2+ SKBR3 and AU565 BC cells and their LTR derivatives were used. Changes in cell growth upon genetic and pharmacologic inhibition of the MVA pathway were quantified by methylene blue staining. Luciferase reporter assays and western blots (WB) measured changes in total and phosphorylated (S127 and S381/inactive) YAP protein levels to examine activity of the YAP/TAZ TF complex. To validate the function of YAP/TAZ in resistance, we performed YAP/TAZ knockdown (siRNA), overexpression of dominant-active YAP constructs (S381A, S381/127A), and qRT-PCR assessment of YAP/TAZ target gene expression.

Results: ZA, like simvastatin (Sim), selectively inhibited the growth of resistant cells in a dose-dependent manner. This inhibition was rescued by geranyl geranyl pyrophosphate (GGPP), a downstream metabolite, but not by MVA, an upstream metabolite, indicating the on-target effect of ZA. Further, ZA and Sim combination showed a synergistic growth-inhibitory effect in R but not in parental (P) cells. YAP/TAZ luciferase reporter assays and phosphorylated YAP and total TAZ levels by WB, confirmed the increased activity of YAP/TAZ in R models, which was selectively inhibited by Sim or ZA and was rescued by the corresponding downstream metabolites. YAP/TAZ knockdown selectively inhibited resistant cell growth and mTOR signaling in R vs. P cells, and dominant-active YAP/TAZ rescued the mTOR inhibition by Sim. YAP/TAZ inhibition by siRNA or by Sim significantly decreased the expression of YAP/TAZ target gene survivin in R vs. P cells, and the Sim inhibition was rescued by MVA.

Conclusions: The MVA pathway plays a critical role in mediating resistance to anti-HER2 therapy, which was overcome by Sim and ZA either alone or in combination. Given the synergistic effect of Sim and ZA, their combination may offer a therapeutic strategy to overcome HER2-targeted therapy resistance. Our results also reveal the role of YAP/TAZ in MVA pathway-mediated HER2-targeted therapy resistance, which could suggest new biomarkers and therapeutic targets.
Title: AP-1 as a potential mediator of resistance to the cyclin-dependent kinase (CDK) 4/6-inhibitor palbociclib in ER-positive endocrine-resistant breast cancer

Body: Background: The CDK4/6-inhibitor palbociclib (Palbo) in combination with endocrine therapy (ET) substantially improves progression-free survival compared to ET alone. However, almost all initial responders eventually develop resistance and relapse. Delineating the early adaptive signaling and the mechanisms underlying resistance to CDK4/6 inhibition is therefore crucial to identify new biomarkers and therapeutic targets to enhance the efficacy of Palbo and improve patient outcome.

Materials and Methods: MCF7 parental (P) cells and derivative lines made resistant (R) to tamoxifen (TamR), estrogen deprivation (EDR), or fulvestrant (FulR) were used. The MCF7P line and its endocrine-R (EndoR) derivatives were exposed to increasing concentrations of Palbo to generate acquired Palbo-R (PDR) models. The proteomic/signaling profiles of P and EndoR cells upon short-term Palbo treatment and as PDR develops were determined using reverse-phase protein arrays (RPPA). Transcriptional activity of the activator protein-1 (AP-1) transcription factor (TF) was measured by luciferase reporter assay. Global AP-1 blockade was achieved using a pINDUCER system to express doxycycline (Dox)-inducible dominant-negative (DN) c-Jun that lacks the transcriptional activation domain. Cell growth and colony formation were assessed using methylene blue staining and clonogenic assays, respectively. Levels of phosphorylated (p)-RB and CDK2 were assessed by Western Blot.

Results: In P and all EndoR cell models, Palbo inhibited cell growth and colony formation in a dose-dependent manner, though the inhibitory effect was greater in the EndoR cells compared to P cells [IC50 value of P cells >3 times that of EndoR lines (p<0.001); clonogenic % inhibition at 100nM = 54 in P and >85 in EndoR lines (p<0.001)]. Across the P and all EndoR models, short-term Palbo treatment resulted in increased levels of several membrane and intracellular signaling molecules, TFs, and enzymes. Among these, the AP-1 TF components c-Jun and p-c-Jun showed the highest increase across all models, with the utmost change observed in the TamR model (Fold-change = 4.4, 4.0 for total and p-c-Jun, respectively). Since we also observed that acquired resistance to Palbo in the TamR model was associated with higher AP-1 transcriptional activity and increased total and p-c-Fos levels, we assessed the efficacy of combining Palbo with AP-1 blockade in the TamR model. In two independent TamR clones ectopically expressing inducible DN-c-Jun, AP-1 blockade (+Dox) in combination with Palbo was highly effective in inhibiting cell growth and reducing p-RB and CDK2 levels compared to single agent treatments. In addition, in both the TamR/DN-c-Jun-expressing clones, the combination of Palbo, AP-1 blockade, and fulvestrant resulted in cell death and a significantly greater cell growth inhibition compared to any dual or mono treatments.

Conclusion: Our results suggest activation of AP-1 as a potential mechanism of resistance to Palbo in ER+ EndoR models. Transcriptomic profiling of the Palbo-sensitive and R cells, currently underway, will provide an in-depth understanding of the role of AP-1 as well as other key targets and associated molecular mechanisms in Palbo resistance.
Title: PRKCQ promotes chemotherapy resistance by regulating levels of Bim

Hanna Y Irie¹, Jessica H Byerly¹, Koichi Ito¹ and Elisa R Port². ¹Icahn School of Medicine at Mount Sinai, New York, NY and ²Mount Sinai Hospital, New York, NY.

Body: Background/Rationale: Genomic heterogeneity and lack of targeted therapies pose significant challenges for the treatment of triple negative breast cancer (TNBC), particularly those that are resistant to standard of care chemotherapy treatments. We identified PRKCQ as a driver of epithelial-mesenchymal transition (EMT) that promotes anoikis resistance, invasion and metastatic lung colonization of TNBC cells. PRKCQ also promotes resistance to the apoptosis-inducing effects of chemotherapies standardly used to treat patients with TNBC. Downregulation of PRKCQ enhances apoptosis of chemotherapy-treated TNBC cells. Our study aims to determine the mechanism for chemotherapy sensitivity regulation by PRKCQ and to determine whether PRKCQ is associated with relative chemotherapy resistance in patient triple negative tumors.

Methods: Using models of both gain- and loss-of function of PRKCQ, we performed a comprehensive analysis of the expression status of Bcl2 family members, which are directly responsible for chemotherapy-induced apoptosis. We validated the critical, causal contribution of affected Bcl2 proteins to apoptosis regulation by PRKCQ in TNBC cells. PRKCQ kinase activity-dependent regulation of chemotherapy sensitivity was evaluated using PRKCQ small molecule inhibitors. Finally, an immunohistochemistry-compatible PRKCQ antibody was optimized and is being used to interrogate the expression of PRKCQ in patient triple negative tumor biopsies obtained prior to neoadjuvant chemotherapy treatment.

Results: PRKCQ specifically regulates the expression of Bim, a pro-apoptotic member of the Bcl2 family; overexpression of PRKCQ suppresses Bim transcript and protein levels, whereas PRKCQ downregulation in TNBC cells increases these levels. The kinase activity of PRKCQ is important for Bim regulation, as overexpression of kinase-dead PRKCQ fails to suppress Bim and treatment with a PRKCQ inhibitor phenocopies the effects of PRKCQ shRNA treatment on Bim levels. Basal levels of Bim have been reported to be a critical set point for sensitivity of cancer cells to chemotherapy and targeted therapies. Here, modulation of Bim levels is sufficient to alter chemotherapy sensitivity of TNBC cells. PRKCQ-dependent suppression of Bim is dependent on Erk/MAPK activity. Ongoing work will determine association between PRKCQ expression with chemotherapy sensitivity of patient TNBC.

Conclusions: PRKCQ is a critical regulator of chemotherapy sensitivity via its ability to modulate Bim, a pro-apoptotic Bcl2 family member. The kinase dependency of this regulation highlights the promises of PRKCQ small molecule inhibitors as a potential therapeutic strategy to enhance the anti-tumor effects of chemotherapy treatment for patients with TNBC.
2017 San Antonio Breast Cancer Symposium

Publication Number: P4-03-07

Title: Combined genome-scale CRISPR-Cas9 knockout screening with transcriptome sequencing to identify paclitaxel related drivers in triple negative breast cancer

Bi Lian, Hu Xin and Shao Zhimin. ¹Fudan University Shanghai Cancer Center, China and ²Shanghai Medical College, Fudan University, China.

Body: Background. Triple negative breast cancer (TNBC) is an aggressive subtype of breast cancers, for which the only standard therapeutics is chemotherapy containing Taxol. However, quite a number of TNBC patients cannot get the expected drug response after paclitaxel treatment and the resistant mechanism has not been clear yet. Other than the traditional “genotype-to-phenotype” means, the high-throughput functional screening, such as CRISPR-CAS9 library, selects genes with the phenotype of interest. Here, we combine the novel screening model with the drug-resistant genotype to explore the decisive role in paclitaxel effect.

Methods. Breast cancer cell line MDA-MB-231(231WT) was treated by paclitaxel from 1ug/ml to 5ug/ml to establish a paclitaxel-resistant cell type (231PTX) for transcriptome sequencing. Genome-scale CRISPR-Cas9 sgRNA library was made into lentivirus to affect MDA-MB-231 cells expressed Cas9 protein (231cas9). Then 231cas9-sgRNA was treated by low dose of paclitaxel for 14 days and was read by next generation sequencing. RNA sequencing data was processed to TPM values and sgRNA data to gene ranking and p value. The threshold of “231PTX TPM/231WT TPM” was above 2 or below 1/2 and the gene p value was smaller than 0.05. Biological technology applied in this study includes western blot (WB), immunofluorescence (IF), real time PCR and cell proliferation assay. In vivo, 20 balb/c mouse were injected MDA-MB-231 in situ for tumor formation and were treated with paclitaxel/normal saline for six times.

Results. Crosstalk between these two sequencing data had result of 124 genes related to paclitaxel resistance (fold change> 2 and p value<0.05 compared Day 14 treated group to Day 14 untreated group) and 18 genes related to paclitaxel sensitivity (fold change< 1/2 and p value<0.05 compared Day 14 untreated group to Day 14 treated group). Considering clinical prognosis and gene information, six paclitaxel resistant candidates and four paclitaxel sensitive candidates were chosen for further research. Eight (STRA6, BIRC3, MTUS1, HDAC9, ADAM28, S1PR5, TNNC1, ZKSCAN7) of ten candidates displayed consistent phenotypes with sequencing results including mRNA expression and the cellular proliferation in paclitaxel treatment. HDAC9 is a histone deacetylation gene that is likely to be a paclitaxel resistant gene. Knockout HDAC9 (231H9 KO) contributed to nearly 2-fold decrease IC50 value (1.7nM versus 3.7nM, p value<0.01). Confocal microscopy observed the formation of multiple spindle foci in the paclitaxel treated 231H9 KO cells. After treatment with paclitaxel, the mark of polymerized tubulin, acetylation tubulin and the mark of cell cycle G2/M, cyclin B1 were notably increased when HDAC9 knockout in both MD-MB1-231 and BT-100 cell lines. In vivo assays found that HDAC9 knockout induced the declined tumorigenesis and more sensitive breast tumors to paclitaxel.

Conclusions. Combined Genome-scale CRISPR-Cas9 knockout screening with transcriptome sequencing is efficient to investigate potent drug targets. In vitro assays suggest that HDAC9 is conductive to paclitaxel resistance in TNBC cells. In vivo results imply inhibition HDAC9 may beneficial to paclitaxel therapeutic response.
2017 San Antonio Breast Cancer Symposium

Publication Number: P4-03-09

Title: Chromosomal instability predicts taxane sensitivity in breast cancer

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Body: Taxanes remain among the most effective agents for the treatment of breast cancer. However, ~50% of patients who receive this therapy do not have a clinical response. Previously, we identified the mechanistic effects of taxane therapy by sampling primary human breast tumors 20 hours after infusion (Sci Trans Med 26:229ra43, 2014). We determined that paclitaxel caused cells to transit mitosis on multipolar spindles, resulting in increased chromosome gains and losses. Preclinical models suggest that tumors have enhanced sensitivity if they have baseline chromosome gains/losses, known as chromosome instability (CIN). The aim of this study was to compare CIN against other predictive biomarkers in a retrospective cohort of advanced breast cancer patients who receive single-agent taxane therapy.

Methods: We identified 36 subjects with metastatic breast cancer and measurable disease who received taxane therapy and had available archived tissue. Responses were determined by RECIST 1.1 criteria. We evaluated chromosome number on a cell-by-cell basis using 6 centromeric FISH probes. Additionally we measured rates of proliferation by phospho-histone H3 (p-HH3) and Ki67, previously reported biomarkers, β-tubulin III, P-gp1 and MAD1 by quantitative immunofluorescence. CIN was estimated as the fraction of cells with non-modal chromosome numbers across chromosomes 3, 4, 7, 9, 10, and 17.

Results: Of the 36 subjects, 19 had ER/PR+HER2- disease (53%), 9 had HER2-positive disease (25%), and 8 TNBC (22%). Single-agent chemotherapy was used for all including paclitaxel in 16, nab-paclitaxel in 17, and docetaxel in 3 (HER2+ patients received concurrent trastuzumab). RECIST responses ranged from disease progression (8%, n=3), stable disease (50%, n=18), and partial response (42%, n=15). No complete responses were observed. Time on therapy ranged from 1.4 months to 28 months. No statistically significant correlations were found between tumor type or prior chemotherapy and response to taxanes. Archived metastatic samples were available for 21 subjects. Analysis showed large variations in Ki67, pHH3, β-tubulin III, P-gp1, MAD1, and CIN amongst samples. The strongest correlation was found between tumor response and high levels of CIN, with a Spearman’s correlation coefficient of 0.38 (p=0.04). Surprisingly, there was an inverse correlation between Ki67 and taxane response, although this did not reach statistical significance.

Biomarkers correlated with response to taxane

<table>
<thead>
<tr>
<th>N=21</th>
<th>Ki67 (%)</th>
<th>pHH3 (H)</th>
<th>βtub3 (H)</th>
<th>P-gp1 (H)</th>
<th>MAD1 (H)</th>
<th>CIN (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>min</td>
<td>0.85</td>
<td>0.00</td>
<td>0.67</td>
<td>3.00</td>
<td>2.83</td>
<td>24.2</td>
</tr>
<tr>
<td>max</td>
<td>45.1</td>
<td>4.89</td>
<td>151</td>
<td>299</td>
<td>231</td>
<td>65.8</td>
</tr>
<tr>
<td>Spearman rho</td>
<td>-0.365</td>
<td>0.242</td>
<td>-0.165</td>
<td>0.232</td>
<td>-0.0896</td>
<td>0.382</td>
</tr>
<tr>
<td>p (1-tailed)</td>
<td>0.0519</td>
<td>0.140</td>
<td>0.237</td>
<td>0.155</td>
<td>0.350</td>
<td>0.0425</td>
</tr>
</tbody>
</table>

H = H-score; CIN is estimated as % of cells with non-modal chromosomes

Conclusions: Chromosomal instability is a promising biomarker for predicting sensitivity to taxane therapies. Additional studies will be necessary to validate CIN as a biomarker and to determine whether 6-chromosome FISH can be supplanted by low-pass single-cell DNA sequencing.
Title: Clinical observation of lapatinib versus continued use of trastuzumab for trastuzumab-resistant HER2-positive advanced breast cancer

Yongmei Yin¹, Wei Li¹, Xiang Huang¹, Jian Wang², Ziyi Fu² and Jun Li¹. ¹The First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, China and ²Nanjing Medical University, Nanjing, Jiangsu, China.

Body: Objective: To compare the efficacy, adverse effects and survival of lapatinib versus continued use of trastuzumab for trastuzumab-resistant human epidermal growth factor receptor 2 (HER2)-positive advanced breast cancer.

Methods: Patients who began the regimen of lapatinib plus capecitabine (LC or LX) or trastuzumab beyond progression (TBP) between May 2013 and October 2016 were selected from the First Affiliated Hospital of Nanjing Medical University. All of their clinical data were recorded, including age, performance status, hormone receptor status, metastatic site, primary or acquired trastuzumab resistance, previous treatment and so on. They were followed up until death or April 30, 2017. The primary end point was progression-free survival (PFS). The efficacy and safety of the two regimens were evaluated according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 and Common Terminology Criteria for Adverse Events (CTCAE) version 4.0, respectively. Data were analyzed by SPSS version 19.0.

Results: In total, 95 patients were identified, including 40 treated with LX and 55 with TBP. Median follow-up time was 16.0 months. There was no difference in objective response rate (ORR) and disease control rate (DCR) between the two groups. By the end of follow-up, median PFS was 6.3 months with LX and 7.1 months with TBP (P=0.676). In patients with primary trastuzumab resistance, longer PFS was observed in LX group compared with TBP group [median PFS: 8.0 months vs. 5.3 months, hazard ratio (HR)=0.416, 95% confidence interval (CI): 0.177-0.981, P=0.038]. The incident rate of new brain metastases during treatment was 2.5% in LX group and 10.9% in TBP group, respectively (P=0.233). Both regimens showed similar outcomes in baseline brain metastases. Grade 3-4 adverse effects included diarrhea 7.5%(3/40) and hand-foot syndrome 12.5%(5/40) in LX group, along with leukopenia 18.2%(10/55), thrombocytopenia 9.1%(5/55) and nausea/vomiting 3.6%(2/55) in TBP group.

Conclusion: LX and TBP were similarly effective for patients with HER2-positive breast cancer progressing on prior trastuzumab-containing therapy. Both were well tolerated in general. Lapatinib tended to reduce the risk of disease progression in patients resistant to trastuzumab primarily. LX or TBP after local treatment appeared to show no difference in treating patients with brain metastases.
2017 San Antonio Breast Cancer Symposium

Publication Number: P4-03-12

Title: Effects of PI3K inhibitors on endocrine-resistant cell lines and differences in the characteristics of ER positive breast cancer cells after acquired resistance to the inhibitors

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Body: Background

Mutations in phosphatidylinositol 3-kinase (PI3K), which encodes the catalytic subunit of PI3Kα, is one of the most frequent genomic alterations and is found in about 40% of estrogen receptor (ER)-positive, HER2 negative breast cancers. PIK3CA mutations promote the growth and proliferation of cancers via activation of the PI3K/mammalian target of rapamycin pathway and can mediate resistance to endocrine therapies in breast cancer. Although several clinical trials for PI3K inhibitors (PI3Kis) in ER-positive metastatic breast cancers are ongoing, the effects of PI3Kis on endocrine-resistant breast cancers with various characteristics and definitive biomarkers of PI3Kis are unclear. Using endocrine-resistant cells established in our laboratory, we evaluated the efficacy of PI3Ki in these cell lines and identified the characteristics associated with acquired resistance to PI3Kis in endocrine-resistant cells.

Results

Long-term estrogen deprivation-resistant (EDR) cell lines and fulvestrant-resistant cell lines (MFR and TFR) were established from MCF-7 and T-47D cells in our previous studies. These cell lines showed different ER expression levels, including high expression (EDR-1), low expression (EDR-2, -3), or no expression (MFR, TFR); all of these cell lines had the same PIK3CA mutations as the parental cell lines. The pan-class1 PI3Ki buparlisib (BKM120) and α-specific PI3K inhibitor alpelisib (BYL719) inhibited the proliferation of endocrine-resistant cell lines when compared with their parental cells. Among endocrine-resistant cells, MFR cells were dramatically inhibited by PI3Kis. Colony formation assays indicated that MFR cells were more sensitive to PI3Kis than other cells lines. Next, we established PI3Ki- and everolimus-resistant cell lines from EDR-1, EDR-2, and MFR cells using BKM120, BYL719, and everolimus. Analysis of the time required to generate resistant cells showed that MFR cells required twice as long to acquire resistance compared with EDR cells. Furthermore, the time required to acquire resistance to BYL719 was shorter than that for BKM120. BYL719-resistant (BYL-R) cells were effectively inhibited by BKM120 to a degree similar to that of parental cells; however, BYL-R cells lost sensitivity to BYL719 and everolimus. Evelolimus-resistant (EVE-R) cells were also the same. In contrast, BKM120-resistant (BKM-R) cells showed less sensitivity to BKM120, BYL719, and everolimus. In other words, the pan-PI3Ki BKM120 was able to inhibit the growth of BYL-R and EVE-R cells, whereas BYL719 and everolimus were not able to inhibit BKM-R cells sufficiently. In addition, there were no changes in ER expression in EDR-1, EDR-2, and MFR cells exposed to PI3Kis for 1 h. Interestingly, ER expression on EDR-2 cells with acquired resistance to PI3Kis was increased compared with that in parental cells.

Conclusion

Our findings showed that PI3Kis exhibited remarkable efficacy in all types of EDR cells, particularly in fulvestrant-resistant cell lines. In PI3Ki- and everolimus-resistant cell lines, BKM120 and BYL719 showed different effects, and BYL719 and everolimus may exhibit cross-resistance. Furthermore, PI3Kis were likely to change the expression of ER.
Body: The majority of breast cancer-specific deaths occur in women with recurrent, ERα (+), metastatic tumors that are initially responsive to endocrine therapy yet become endocrine-resistant. There is a critical need for novel therapeutic approaches to re-sensitize recurrent ERα (+) tumors to endocrine therapies. The objective of this study was to elucidate mechanisms of improved effectiveness of combined targeting of ERα and XPO1, a nuclear transport protein in overcoming endocrine resistance. Using Cignal Finder pathway profiling, Seahorse metabolic profiling and GC/MS whole metabolite profiling, we found that combination of 4-OH-Tamoxifen (4-OH-Tam) and Selinexor (SXR), a highly selective XPO1 inhibitor that is currently in clinical trials for leukemia and prostate cancer, inhibited Akt phosphorylation by changing the phosphorylation status and localization of the kinase. Since we observed dramatic changes in Akt activity, we hypothesized that metabolic pathways and consequently metabolic profile of breast cancer cells would change in the presence of 4-OH-Tam and SXR. These cells were more dependent on mitochondria for energy production. Their glucose and fatty acid dependency decreased in the presence of SXR and cells were more dependent on glutamine as the mitochondrial fuel source. We demonstrated that combined targeting of XPO1 and ERα rewires metabolic pathways and induce autophagy. Remodeling metabolic pathways to regenerate new vulnerabilities in endocrine-resistant tumors is novel, and given the need for better strategies for improving therapy response of relapsed ERα (+) tumors, our findings show great promise for combined targeting of ERα and XPO1 in the clinic to reduce cancer recurrences.
2017 San Antonio Breast Cancer Symposium

Publication Number: P4-03-14

**Title:** OTULIN promotes resistance and metastasis upon chemotherapy in TNBC by activating Wnt/β-catenin signaling

Zhaohui Wu¹, Wei Wang¹, Bo Zhang¹, Jingyan Xue², Yayun Chi² and Jiong Wu². ¹The University of Tennessee Health Science Center, Memphis, TN and ²Fudan University Shanghai Cancer Center, Shanghai, China.

**Body:** Triple-negative breast cancer (TNBC) or Basal-like breast cancer is characterized by worse prognosis than other breast cancer subtypes due to the rapid-arising therapeutic resistance accompanied by aggressive metastasis. The therapy-resistant tumor cells, including cancer stem-like cells (CSCs) with intrinsic resistance or tumor cells acquired resistance during treatments, could repopulate the primary tumors and serve as seeds for recurrent metastatic lesions. However, the molecular events orchestrating the innate and acquired therapeutic resistance are not completely understood. We found a recently identified linear ubiquitin-specific deubiquitinase OTULIN is overexpressed in breast tumors, especially in the basal-like subtype, compared with normal breast tissues. Moreover, OTULIN overexpression significantly increased TNBC cells resistance to chemotherapy and is associated with shorter RFS in breast cancer patients. Mechanistically, we found genotoxic treatments activate OTULIN, which promotes epithelial-to-mesenchymal transition (EMT) in TNBC cells through increased activity of Wnt/β-Catenin signaling. OTULIN overexpression significantly increased TNBC cells resistance to chemodrugs while inhibiting Wnt/β-Catenin sensitized TNBC xenografts to Doxorubicin treatment in vivo. Our findings suggest that OTULIN-mediated Wnt/β-Catenin activation may increase TNBC therapeutic resistance and aggressiveness by promoting EMT. Targeting OTULIN and the chemotherapy-induced Wnt/β-Catenin activation may serves as promising strategy to mitigate drug resistance and reduce metastasis in TNBC patients receiving chemotherapy.
Title: Targeting Src kinase blocks development of afatinib resistance in HER2-positive breast cancer

Neil Conlon¹, Alexandra Canonici¹, Clare Morgan², Mattia Cremona², Bryan T Hennessey², Alex Eustace², Neil O'Brien³, Dennis Slamon³, John Crown⁴ and Norma O'Donovan¹. ¹National Institute for Cellular Biotechnology, Dublin City University, Dublin, Ireland; ²Royal College of Surgeons in Ireland, Dublin, Ireland; ³University of California Los Angeles, Los Angeles and ⁴St. Vincent's University Hospital, Dublin, Ireland.

Body: Background: Afatinib is an irreversible pan-HER inhibitor approved for non-small cell lung cancer. We have previously shown that afatinib inhibits growth of HER2-positive breast cancer cells and enhances response to trastuzumab. However, we have also shown that long-term exposure to tyrosine kinase inhibitors leads to the development of acquired resistance. To determine if acquired afatinib resistance develops in HER2-positive breast cancer cells, we exposed a HER2-positive breast cancer cell line to afatinib for 6 months and investigated alterations in the cells following long-term exposure.

Methods: SKBR3 cells were treated with 150 nM afatinib twice-weekly for 6 months. Growth response to drug inhibitors was assessed by acid phosphatase assay. Drug sensitivity was examined in four HER2-positive cell lines (SKBR3, EFM192A, BT474 and HCC1954) and three acquired trastuzumab resistant cell lines (SKBR3-T, BT474-T and EFM192A-T). Reverse phase protein array (RPPA) was used to determine alterations in key signaling pathways. Src, p-Src, EGFR, p-EGFR, ERK1/2, p-ERK 1/2 levels were examined by Western blotting. To examine the prevention of the development of afatinib resistance, cells were treated twice weekly with afatinib, dasatinib, or the combination and stained with crystal violet when confluent.

Results: Following 6 months of afatinib treatment, the SKBR3-A cells were more resistant to afatinib compared to parental cells (IC₅₀ SKBR3-A 284 ± 28.2 nM vs SKBR3-Par 10.9 ± 3.4 nM). Furthermore, the resistant cells were cross-resistant to lapatinib, neratinib and trastuzumab. RPPA interrogation of the SKBR3-A cells showed alterations in several pathways, including significantly increased levels of p-Src (Y416). SKBR3-A cells were more sensitive to Src inhibition with dasatinib compared to SKBR3-Par cells and the combination of afatinib and dasatinib was highly synergistic in SKBR3-A cells (CI value = 0.09 ± 0.06). The combination of afatinib and dasatinib was also synergistic in the trastuzumab resistant SKBR3-T cells (Table). Afatinib and dasatinib inhibited EGFR and Src activation and ERK 1/2 signalling in SKBR3-A cells.

Short-term resistance assays showed that the addition of dasatinib to afatinib blocks the emergence of resistant cells in three of four HER2 positive cell lines tested and two of the three acquired trastuzumab resistant cell lines tested.

Conclusion: HER2-positive breast cancer cells that are highly sensitive to afatinib can develop acquired resistance to afatinib within six months. Src is a potential target to prevent the development of afatinib resistance and thus combined treatment with afatinib and dasatinib may be beneficial in patients with HER2-positive breast cancer.

<table>
<thead>
<tr>
<th></th>
<th>20 nM Afatinib</th>
<th>40 nM Dasatinib</th>
<th>20 nM Afatinib + 40 nM Dasatinib</th>
</tr>
</thead>
<tbody>
<tr>
<td>SKBR3</td>
<td>25.2 ± 6.1</td>
<td>109.9 ± 6.2</td>
<td>22.6 ± 6.2</td>
</tr>
<tr>
<td>SKBR3-A</td>
<td>62.1 ± 0.7</td>
<td>87.3 ± 11.0</td>
<td>36.3 ± 0.3*</td>
</tr>
<tr>
<td>SKBR3-T</td>
<td>42.6 ± 4.9</td>
<td>107.4 ± 9.7</td>
<td>29.3 ± 3.5*</td>
</tr>
<tr>
<td>BT474</td>
<td>18.2 ± 3.0</td>
<td>79.5 ± 5.2</td>
<td>13.3 ± 1.1</td>
</tr>
<tr>
<td>BT474-T</td>
<td>6.9 ± 2.7</td>
<td>95.3 ± 2.0</td>
<td>6.2 ± 2.3</td>
</tr>
<tr>
<td>EFM192A</td>
<td>39.1 ± 4.7</td>
<td>105.7 ± 3.1</td>
<td>35.3 ± 2.7</td>
</tr>
<tr>
<td>EFM192A-T</td>
<td>30.4 ± 5.2</td>
<td>99.8 ± 2.5</td>
<td>28.5 ± 4.4</td>
</tr>
<tr>
<td>HCC1954</td>
<td>61.8 ± 8.1</td>
<td>80.5 ± 8.2</td>
<td>19.4 ± 3.6*</td>
</tr>
</tbody>
</table>

* indicates enhanced anti-proliferative response for the combination compared to the single agents.
Title: Cyclin E affects Smad3 pathway in trastuzumab resistant HER2+ breast cancer

Joseph T Decker¹, Lei Wan¹, Lonnie D Shea¹ and Jacqueline S Jeruss¹. ¹University of Michigan.

Body: Introduction and Objective: HER2 amplification is found in approximately 20% of patients with breast cancer and is associated with poor prognosis. Trastuzumab, a humanized anit-HER2 antibody, has been shown to improve patient outcomes, but clinical efficacy for this treatment is limited by the high rate of drug resistance. Cyclin E overexpression and cyclin-dependent kinase 2 (CDK2) activation correlate with trastuzumab resistance, and the underlying mechanisms of this correlation are an area of active study. In this work, we examined the contribution of cyclin E/CDK2-mediated Smad3 noncanonical phosphorylation to trastuzumab resistance in HER2+ breast cancer.

Methods: Trastuzumab resistant BT474R2 cells were developed by culturing BT474 cells in trastuzumab-supplemented media for 18 months. These cells were then inoculated into immunodeficient mice and treated with trastuzumab twice weekly. BT474 and BT474R2 cells were used to study cell proliferation, phosphorylation in the Smad3 linker region and transcription factor activities with a living cell array.

Results: Inhibition of cyclin E activity with CDK2 inhibitor therapy (CDK2i, 600 nM) significantly decreased BT474R2 cell proliferation. CDK2i treatment led to decreased phosphorylation at T179 (p<0.01), S204 (p<0.01) and S213 (p<0.05) in the Smad linker region. Further, CDK2i increased expression of p15 (p<0.05) and decreased expression of c-myc (p<0.05) in comparison with trastuzumab treatment. Transduction of BT474R2 cells with a 5M Smad3 construct containing inhibitory mutations in 5 CDK2 phosphorylation sites resulted in decreased cell proliferation. In a transfected cell array using BT474 and BT474R2 cells, activities of cell proliferation and metastasis-associated transcription factors were significantly different between the trastuzumab sensitive and resistant cells.

Conclusions: Taken together, overexpression of cyclin E leads to Smad3 noncanonical phosphorylation and results in trastuzumab resistance in HER2+ breast cancer. CDK2i treatment may be a promising therapeutic strategy for patients with trastuzumab resistance.
Title: Loss of mismatch repair predicts resistance to endocrine therapy and sensitivity to CDK4/6 inhibitors in ER+ breast cancer

Svasti Haricharan1, Nindo Punturi1, Purba Singh1, Kimberly R Holloway1, Meenakshi Anurag1, Jacob Schmelz1, Cheryl Schmidt1, Jonathan T Lei1, Vera Suman2, Kelly Hunt2, John A Olson Jr4, Jeremy Hoog5, Shunqiang Li5, Shixia Huang1, Dean P Edwards1, Shyam M Kavuri1, Matthew N Bainbridge6, Cynthia X Ma5 and Matthew J Ellis1. 1Baylor College of Medicine; 2UT MD Anderson Cancer Center; 3Mayo Clinic; 4University of Maryland School of Medicine; 5Washington University School of Medicine and 6Rady's Children's Hospital.

Body: Estrogen receptor positive (ER+) breast cancer is treated with endocrine therapy but intrinsic resistance occurs in ~1/3 of patients and acquired resistance in ~1/5 of the remainder. While many resistance mechanisms have been explored, clinical trials testing associated therapeutics have had mixed outcomes. Understanding mechanisms of resistance and finding therapeutic strategies to target them, therefore, remain important challenges.

In this study we discover an intriguing link between mismatch repair (MMR) deficiency, specifically of the MutL complex (MLH1/3, PMS1/2), and endocrine therapy resistance in ER+ disease. We find a direct role for MutL loss in inducing endocrine therapy resistance in vitro and in vivo by knocking down multiple MutL genes using CRISPR and stable shRNA approaches validated using standard rescue experiments. We identify the underlying mechanism: MutL deficiency in ER+ breast cancer abrogates Chk2-mediated feedback inhibition of CDK4/6 that appears necessary for endocrine therapy responsiveness. Consequently, pharmacological targeting of CDK4/6 in vitro and in vivo significantly inhibits growth of endocrine therapy resistant MutL-deficient ER+ breast cancer cells. These results are corroborated by data from a neoadjuvant clinical trial demonstrating that cell cycle regulation of MutL-mutant tumors is estrogen-independent but CDK4/6 dependent.

The results of this study provide important biological and clinically relevant insights. 1) a novel role for MMR in endocrine therapy resistance 2) A mechanism underlying the effectiveness of CDK4/6 inhibitors in some de novo endocrine therapy resistant tumors. While there are currently no biomarkers to guide the use of CDK4/6 inhibitors for ER+ breast cancer, markers of MMR dysregulation could identify patients in whom CDK4/6 inhibition may, most effectively, prevent disease recurrence.
Characterising the effects of neoadjuvant endocrine therapy on primary cancers and nodal metastasis

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Background: Approximately 40% of ER+ breast cancer present with nodal metastasis. To date, there has been no comparison of the molecular response of primary cancers and metastases to ET. Recent evidence suggests that nodal metastases have different clones and subclones compared to the primary tumour. The aim of this study is to characterise the molecular response of primaries and nodal metastases to ET.

Methods: A unique set of 7 post-menopausal women with ER-positive breast cancer had biopsies taken from the primary tumour and a positive lymph node at diagnosis and at surgery following 3-12 months of neoadjuvant letrozole. 14-day and 3-6 month on-treatment biopsies from the primary tumour and involved nodes were also taken from the same patients, giving a total of 75 samples. Lymph node FFPE blocks were stained for cytokeratin and macro-dissected to enrich for tumour tissue. RNA and DNA were extracted and Ribo0-RNAseq, DNA exome sequencing and somatic mutation detection using UNCeqR performed. Whole-transcriptome AmpliSeq targeted-sequencing has been analysed for 4 patients.

Results: Multi-dimensional scaling and hierarchical clustering analysis based on all transcripts and the 500 most variably expressed genes revealed that primaries and nodal metastases are strongly associated at diagnosis but some nodes diverge during ET treatment. Analysis of estrogen-responsive proliferation-associated genes (n=60) in nodal metastasis revealed a reduction in expression of the majority of genes with ET. However, the expression levels of some remained high in the on-treatment node samples in all 4 patients analysed compared with the matched primary tumour on treatment. In particular, expression of genes involved in DNA replication and regulation of cell cycle including MCM6 and RRM2 (DNA replication), ASPM and CEP55 (mitosis) and CDKN3 (regulation of cell cycle) persisted at high levels in nodal metastases, but reduced in the primary cancers. Similarly, primary tumours had increased levels of ECM remodeling genes (n=60) as treatment continued, while levels in the nodal metastasis were heterogeneous on-treatment. Full genome sequencing results will be available by December 2017.

Discussion
- This is the first study to investigate genomic and transcriptomic changes with ET in both primary cancers and nodal metastases.
- On-treatment changes in nodal disease are heterogeneous between patients and within the same patient.
- Nodal metastases do respond to ET with reduced levels of proliferation-associated genes.
- Some proliferation-associated genes appear to maintain higher expression in nodal disease.
- Patterns of gene expression observed in some nodal metastases are consistent with profiles previously described by us for ET resistance and recurrent disease.
- Nodal metastases may accumulate mutations during treatment with ET and on-going analysis will clarify this.
Title: Hyperactive FOXA1 activates super-enhancer-engaged HIF2α/EPAS1 to promote endocrine-resistant metastatic ER-positive breast cancer

Xiaoyong Fu\(^1\,^2\,^3\), Resel Pereira\(^1\,^2\), Carmine De Angelis\(^1\,^2\,^4\), Jamunarani Veeraraghavan\(^1\,^2\,^4\), Martin J Shea\(^1\,^2\,^4\), Sarmistha Nanda\(^1\,^2\,^4\), Qin Feng\(^3\), Rinath Jeselsohn\(^5\), Bert W O'Malley\(^2\,^3\), Myles Brown\(^5\), C Kent Osborne\(^1\,^2\,^3\,^4\) and Rachel Schiff\(^1\,^2\,^3\,^4\).

1 Lester and Sue Smith Breast Center, Baylor College of Medicine, Houston, TX; 2 Dan L. Duncan Comprehensive Cancer Center, Baylor College of Medicine, Houston, TX; 3 Baylor College of Medicine, Houston, TX; 4 Baylor College of Medicine, Houston, TX and 5 Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA.

Body: Background: We have recently reported that acquired endocrine resistance (Endo-R) in multiple ER+ breast cancer (BC) Endo-R cell models is driven by high levels of FOXA1 (High-FOXA1), via gene amplification and/or overexpression (OE), leading to coordinated reprogramming of the FOXA1 genomic binding (cistrome) and transcriptome. Forced FOXA1 OE in parental (P) cells induced similar transcriptional reprogramming leading to Endo-R and metastasis. Recent clinical data showing enrichment of FOXA1 amplification in ER+ metastases further support the clinical importance of our findings. However, the molecular components and the mechanism of High-FOXA1-induced transcriptional reprogramming in Endo-R and metastasis are unknown.

Methods: High-FOXA1-containing MCF7 tamoxifen-resistant (TamR) and P/FOXA1-OE cells were used in this study. An integrative multi-OMICS approach was employed to analyze transcriptome (RNA-seq), FOXA1 cistrome, and histone H3K27 acetylation (ac) (ChIP-seq). Intersection of High-FOXA1-induced transcriptome and distinct FOXA1 cistrome-predicted genes defined a High-FOXA1 core gene signature (CGS). Gene Set Enrichment Analysis (GSEA) and Gene Ontology (GO) were used for functional annotation. Cell growth and migration/invasion were measured by a bright-field automated cell counter and Transwell insert system. Altered gene expression was measured by RT-qPCR. High-FOXA1 signaling inhibition included gene knockdown (siRNA) or pharmacologic blockage (the EPAS1 inhibitor PT2385). The predictive role of EPAS1 and the associated gene signature were analyzed using publicly available BC datasets.

Results: FOXA1 OE reprogrammed the FOXA1 cistrome in P cells to resemble that of the TamR cells. The FOXA1 cistrome was significantly correlated with the deposition of H3K27ac in TamR vs. P cells (P<2.2e-16). Similarly, the differentially expressed genes in TamR vs. P cells were enriched for FOXA1 binding at enhancers demarcated by H3K27ac (P=8e-125). The FOXA1-CGS was linked to multiple metastasis-related GO terms including “hypoxia response”, enriched for the cancer secretome gene set (P=4.1e-16), and highly represented in the Endo-R transcriptome across our multiple cell models (MCF7, 600MPE, and CAMA1) (P<0.01). Integrative analysis of H3K27ac-defined super-enhancers (SEs) and altered cistrome/transcriptome upon High-FOXA1 nominated EPAS1, a hypoxia-inducible transcription factor (TF), as a top candidate of SE-activated TFs amplifying High-FOXA1 signaling. EPAS1 blockade markedly repressed the secretome genes (e.g., IL8 and S100P) and cell migration and invasion in TamR cells. Primary ER+ tumors (TCGA) with high EPAS1 are enriched for a cancer secretome gene set (P=3e-4). High EPAS1 predicts poor distant metastasis-free survival in ER+ BC treated with endocrine therapy (P=0.34).

Conclusions: High-FOXA1 induces transcriptional reprogramming by coordinating histone enhancer marks to activate EPAS1 via an SE mechanism, which in turn mediates transcriptional reprogramming, partly via inducing a pro-metastatic secretome, to promote Endo-R and metastasis. Targeting the High-FOX1/EPAS1 axis to block transcriptional reprogramming may offer a new therapeutic strategy to prevent and treat Endo-R metastatic ER+ BC.
Title: Identification and development of oral estrogen receptor PROTAC degraders for breast cancer

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Body: ER-positive breast cancers comprise approximately 70-80% of all newly diagnosed cases. Downregulation or degradation of ER is a treatment approach currently used in the clinic to target estrogen receptor signaling. Faslodex, the only clinically-approved ER-downregulator, is administered as a monthly intramuscular injection with limiting pharmaceutical properties. Reasoning that an orally-available estrogen receptor degrader would be beneficial to patients, we have leveraged our experience in targeted protein degradation to generate and characterize novel proteolysis targeting chimeras (PROTACs) against estrogen receptor alpha. PROTACs are heterobifunctional molecules that facilitate the formation of a “trimer complex” comprised of the PROTAC, a pathogenic target protein of interest and an E3 ligase, which catalyzes the ubiquitylation and subsequent degradation of the target protein via the proteasome. To identify novel ER degraders (ER PROTACs), we have used several in vitro assays to characterize the extent of target engagement and receptor degradation. Potent ER PROTACs with good oral exposure and other pharmaceutical properties in multiple pre-clinical species were further evaluated in breast cancer xenograft models. Orally-administered ER PROTACs achieved >80% degradation of estrogen receptor alpha and demonstrated single agent tumor growth inhibition in these disease models. Further, combination with a CDK4/6 inhibitor resulted in the expected improvement in anti-proliferative activity.
Body: Background: ER activation is a major ruler of cell biology in ER+ve breast cancer. Hence, ER dependent gene expression at diagnosis, may unveil most of the oncogenic mechanisms responsible of potential tumour relapse and metastasis. Thus, we hypothesized that oestrogen deprivation through PETx may unveil underlying tumour biology with deeper prognostic implications. To explore this, we studied changes in PAM50 intrinsic subtyping and Risk of Recurrence score throughout PETx and their correlation with known prognostic factors.

Methods: Clinical-pathological data were evaluated in a series of patients with stage I-III ER-positive/HER2-negative breast cancer treated in 6 centers in Spain with PETx during more than two months with available baseline and surgical samples. The expressions of 50 genes were measured in baseline samples and surgical specimens using the nCounter platform. Intrinsic subtypes and Risk of Recurrence score (ROR) were determined by the research-based PAM50 predictor. Response by ultrasonography (US) and magnetic resonance (RMI) between diagnosis and before surgery and PEPI score in surgical samples were used as the endpoints. Association between two variables was evaluated using $\chi^2$ test or Pearson correlation. All statistical tests were two-sided and considered significant when $P \leq 0.05$.

Results: Gene expression profile was feasible in 58 pre/post sample pairs with a median of 7.8 months (range 2.5-40.6) of PETx with AIs (98.3%) or tamoxifen (1.7%). At baseline, 68.9%(n=40) were classified as Luminal A, followed by Luminal B (24.1%; n=14), HER2 enriched (HER2-E) (5.2%; n=3) and Normal-like (1.8%; n=1). Radiologic response did not change significantly according to intrinsic subtype either by MRI or US ($P>0.05$). Instead, PEPI score varied according to intrinsic subtype ($P=0.024$). Thirteen (32%) of LumA, while neither of LumB or HER2-E tumours showed a PEPI score Group 1. PETx resulted in changes in the intrinsic subtype in 29 (50%) of tumours

<table>
<thead>
<tr>
<th></th>
<th>Her2-E post</th>
<th>LumA post</th>
<th>LumB post</th>
<th>Normal post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Her2 pre</td>
<td>2 (66.7%)</td>
<td>1 (33.33%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LumA pre</td>
<td>1 (2.5%)</td>
<td>21 (52.5%)</td>
<td>3 (7.5%)</td>
<td>15 (37.5%)</td>
</tr>
<tr>
<td>LumB pre</td>
<td>0</td>
<td>6 (42.86%)</td>
<td>5 (35.71%)</td>
<td>3 (21.43%)</td>
</tr>
<tr>
<td>Normal pre</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (100%)</td>
</tr>
</tbody>
</table>

Of note, 2 of 3 (66.7%) HER2 tumors, and 5 of 14 (35.7%) LumB tumors did not change their profile. Forty-eight (83%) tumours showed a decrease in ROR score after PETx ($P<0.001$). ROR was over 30 in 29 tumours (49.1%) at baseline and 16 (27%) at surgery. Change in ROR was moderately correlated with percentage of change in Ki-67 [correlation: 0.417 ($P<0.01$)]. Correlation of surgical tumour subtype with IHC markers of cell-viability/apoptosis will be presented.

Conclusion: Oestrogen deprivation of luminal tumours through PETx results in profound changes in tumour biology including a migration in intrinsic subtype in 50% of tumours. Correlation of the largely decreased ROR with changes in Ki67 reveals the potential prognostic additional information generated by profiling tumours after PETx. ER-blockade may unveil underlying tumour oncogenic capabilities for relapse, survival and metastasis. Hence, the post-PETx gene expression profile, molecular subtype and ROR may bear incremental prognostic and predictive
information generating a novel scenario for optimal clinical decision making.
Title: AZD5363, an AKT inhibitor, significantly inhibits key biomarkers of the AKT pathway and Ki67, in a randomized, placebo, controlled study (STAKT) in human breast cancers

John FR Robertson¹, RE Coleman³, KL Cheung¹, Abigail Evans¹⁰, Chris Holcombe⁴, Anthony Skene⁸, D Rea¹¹, Samreen Ahmed⁸, A Jahan⁵, S Kelly, Kieran Horgan⁶, Petra Rauchhaus², Roberta Littleford⁴, Andrew Foxley¹⁴, JPO Lindemann¹⁴, Martin Pass¹⁴, Paul Rugman¹⁴, Rahul Deb¹², Pauline Finlay¹⁵ and Julia MW Gee¹⁵.¹University of Nottingham, Nottingham; ²University of Dundee, Dundee; ³University of Sheffield, Sheffield; ⁴Royal Liverpool University Hospital, Liverpool; ⁵King's Mill Hospital, Nottingham; ⁶Leeds General Infirmary, Leeds; ⁷Weston Park Hospital, Sheffield; ⁸Leicester Royal Infirmary, Leicester; ⁹Royal Bournemouth & Christchurch NHS Foundation; ¹⁰Poole Hospital NHS Foundation Trust; ¹¹University of Birmingham; ¹²Royal Derby Hospital; ¹³Derriford Hospital; ¹⁴AstraZeneca and ¹⁵Cardiff University.

Body: Background: AKT is an important intracellular control point through which Type 1 growth factors and IGFR signal. Mutations in PIK3CA, AKT and PTEN are prevalent in estrogen receptor positive (ER+) breast cancer (BC) and have been implicated in resistance to endocrine therapies. AZD5363 is an inhibitor of AKT 1, 2 and 3 currently in Phase 2 trials for BC and other solid cancers.

Design: The study examined whether AZD5363 impacts on key biomarkers within the AKT pathway and their subsequent effects on Ki67, a marker of tumor proliferation. STAKT is a multi-center, two-stage, double blind, randomized, placebo controlled, biomarker 'window-of-opportunity' trial in women with newly diagnosed, previously untreated ER+ BC who were deemed would require chemotherapy as part of their primary treatment regimen. Stage 1 assessed AZD5363 at a dose of 480mg bd p.o. versus matching placebo. Up to 30 patients per arm were permitted, to allow 12 subjects per arm with evaluable paired biopsies - obtained at baseline, and after 4.5 days of AZD5363 / placebo. Primary endpoint markers were pPRAS40, pGSK3β and Ki67 assessed by immunohistochemistry. pPRAS40 and pGSK3β were assessed by H-scores and measured separately for cytoplasmic (cyto), nuclear (nuc) and total (cyto+nuc) staining. Ki67 was assessed as % positive staining of 500 tumor nuclei. Laboratory staff were blinded to treatment arm and whether the biopsies were taken before or after AZD5363/placebo. Changes in marker expression (both absolute and %) between biopsies were calculated, and compared between the two groups. An ANOVA test was applied for normally distributed data and Wilcoxon Mann-Whitney used if not normally distributed.

Results: 28/36 patients were evaluable with patient & tumor characteristics as follows: 17 received AZD5363 and 11 placebo; the median ages were 48 & 49 years respectively. 27 patients were Caucasian and 1 African-American. Tumors were all ER+. For HER2 status 8 were positive & 9 negative in the AZD5363 treated group compared to 2 & 9 respectively in the placebo group. For pPRAS40 and pGSK3β cyto was the predominant staining while for Ki67 staining was nuclear. Changes in each marker with associated p-values are shown in the table.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Type of change vs baseline</th>
<th>Degree of change in AZD5363 arm (n=17)</th>
<th>p-value versus placebo arm (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pPRAS40 (H-score)</td>
<td>Absolute</td>
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<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total</td>
<td>%</td>
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<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cyto</td>
<td>Absolute</td>
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</tr>
<tr>
<td>Cyto</td>
<td>%</td>
<td>-55.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Nuc</td>
<td>Absolute</td>
<td>+6.9</td>
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</tr>
<tr>
<td>Nuc</td>
<td>%</td>
<td>+8.9</td>
<td>0.94</td>
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<tr>
<td>pGSK3β (H-score)</td>
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</tr>
<tr>
<td>Total</td>
<td>%</td>
<td>-39.0</td>
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<tr>
<td></td>
<td>Absolute</td>
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<tr>
<td>--------</td>
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</tr>
<tr>
<td>Cyto</td>
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<td>Nuc</td>
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<td>Nuc %</td>
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<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Ki67 (% cells+)</td>
<td>-9.6</td>
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<td>0.031</td>
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<tr>
<td>%</td>
<td>-29.4</td>
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<td>0.052</td>
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</tbody>
</table>

**Conclusions**

- AZD5363 for 4.5 days caused highly significant falls in pGSK3β and pPRAS40, key markers of AKT pathway activation.
- AZD53643 also caused a significant decline in Ki67 even after only 4.5 days of drug. This is one of the shortest ‘window’-studies to report such an early effect on proliferation.
- Placebo controlled ‘window’ studies of this short duration can provide important evidence of the therapeutic potential early in a drug’s development.
Title: Characterization of the effects of estrogen receptor alpha Y537S and D538G mutations on receptor function and pharmacology

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Body: The frontline therapy for estrogen receptor alpha (ERα) positive Breast Cancer (ER+BC) involves various forms of endocrine therapy, consisting of either Selective Estrogen Receptor Modulators (SERMs) or aromatase inhibitors. An emerging mechanism of ER+BC resistance to endocrine therapy, and consequently disease relapse, has been associated with a set of “hotspot” mutations in and near to helix-12 of the ERα ligand binding domain. Selective Estrogen Receptor Degraders/Down-regulators (SERDs), such as GDC-0810, AZD9496 and GDC-0927, represent a current major pharmacological strategy being applied to develop treatments for such resistant ER+BC. Here, we compare 2 of the most frequent ERα hotspot mutations (Y537S and D538G), with ERα wildtype (WT) and the ability of a set of ERα ligands (including GDC-0810, AZD9496 and GDC-0927) to bind, antagonize and degrade ERα. The concentration of each drug required to bind, antagonize or degrade ERα Y537S or ERα D538G was typically higher than that required for ERα WT. Importantly, ERα Y537S is resistant to estradiol stimulated protein degradation and 4-hydroxy-tamoxifen (a major active metabolite of tamoxifen) stabilizes ERα Y537S protein. This represents a potential mechanism of resistance of ERα Y537S ER+BC to Tamoxifen therapy.
Title: Therapeutic strategy for ERα mutation driven-endocrine resistance in ER-positive breast cancers

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Body: Background: Although estrogen receptor (ER)-positive breast cancers are treated with endocrine therapy, 25% of these patients are at risk of relapse and the development of acquired endocrine resistance. Recently mutations in the ER gene (ESR1) have been identified which induce resistance to endocrine therapy. The most frequent ESR1 mutation, Y537S, promotes ligand-independent ER activity. It is known that ER regulates the cell cycle in a ligand-dependent manner. In this study, we examined the effects of the Y537S ESR1 mutation on cell cycle signaling and therapeutic response to checkpoint inhibitor.

Material and Methods: MCF-7 cells expressing the Y537S ESR1 mutation were generated by CRISPR-Cas9 knock-in techniques. Cells were incubated in phenol red minus medium containing 5% charcoal-dextran treated serum for 5 days to remove exogenous hormones. Cell cycle and apoptosis were examined by Flow cytometry and Annexin-V assays. Proliferation was analyzed by BrdU incorporation. Cell senescence was determined using beta-galactosidase assays. Cell cycle checkpoint kinases were examined by western blot analysis. Cell growth was analyzed using soft agar or MTT assays.

Results: Levels of p53 and apoptosis pathway proteins were significantly elevated in Y537S ESR1 mutant cells using RNA expression and reverse-phase protein microarrays. The ATM/ATR and Chk1/Chk2 mediated checkpoint signaling, the upstream pathway of p53, was activated in ESR1 Y537S mutation, which was repressed with fulvestrant treatment. ESR1 Y537 mutant cells accumulated about 5 fold in S phase and 1.7 fold in G2/M phase compared to control cells in estrogen deprived (ED) condition. BrdU incorporation was also increased about 2.5 fold compared with parental cells in estrogen-free medium. In addition, ESR1 Y537 mutant cells expressed a DNA double-strand break marker, gamma-H2AX protein in ED condition. Apoptosis and senescence were observed in ESR1 Y537S mutant cells in regular medium, however, apoptosis was not shown in ED medium. Chk1 inhibitor, PF477736 sensitized MCF-7 expressing ESR1 Y537S mutation to endocrine treatments such as fulvestrant, tamoxifen and AZD9496.

Conclusion: Combination therapy with cell cycle checkpoint kinase inhibitor may lead better prognosis in ER mutation driven-endocrine resistance in postmenopausal breast cancers.
Title: New oral SERD elacestrant (RAD1901) shows efficacy in breast cancer models harbouring ESR1 mutations and enhances the antiproliferative activity of mTORC1 and CDK4/6 inhibitors

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Body: Background: Targeting estrogen receptor (ER) signalling is the main therapeutic option for ER+ breast cancer (BC). However, over 30% of patients relapse with endocrine resistance emphasising the need for improved therapeutic strategies. The prevalence of ESR1 mutations in relapsed tumours highlights the sustained reliance on ER signalling, rationalising continued targeting of ER. Unlike other endocrine therapies such as aromatase inhibitors (AI) and tamoxifen, selective ER degraders (SERDs) are competitive ER antagonists, that induce a conformational change in ER resulting in ubiquitination and degradation via the proteasomal pathway. Fulvestrant has shown clinical utility in advanced BC but is limited by its pharmaceutical properties highlighting the need for SERDs with enhanced bioavailability and pharmacokinetic properties. Here, we show that elacestrant (RAD1901) an orally bioavailable SERD, has antitumor activity in endocrine sensitive and resistant models of ER+ BC. Furthermore, elacestrant enhances the efficacy of mTORC1 inhibitor, everolimus and CDK4/6 inhibition, in model systems.

Methods: Several ER+ BC lines adapted to long-term E deprivation (LTED) and harbouring wild-type or a naturally occurring ESR1 mutation, were treated with elacestrant or fulvestrant +/- estradiol (E2). Effects on cell proliferation, cell signalling, cell cycle, transcription, ER protein stability and ER genomic binding were assessed. Efficacy in combination with everolimus, palbociclib and abemaciclib was also evaluated.

Results: Cell proliferation assays in 2D and spheroids in the presence of 0.01nM E2 showed a concentration-dependent decrease in proliferation in response to elacestrant and fulvestrant. GI₅₀ values for elacestrant in general were 10-fold higher than fulvestrant but equated to doses that are clinically achievable for the drug. Most importantly, elacestrant suppressed proliferation of two LTED models harbouring ESR1 mutations, MCF7 LTED⁵³⁷C (GI₅₀ 5nM) and SUM44-LTED⁵³⁷S (GI₅₀ 100nM). GI₅₀ values of elacestrant and fulvestrant showed similar reduction of ER, progesterone receptor (PGR) and cyclinD1 together with decreased phosphorylation of retinoblastoma (RB), concordant with cell cycle arrest. Chromatin immunoprecipitation (ChIP) for ER in response to elacestrant or fulvestrant showed a reduction in recruitment of ER to TFF1, GREB1 and PGR promoters and concomitant reduction in mRNA expression of these genes in the presence of E2. Elacestrant-mediated ER depletion was dependent on the 26S proteasome, as addition of the proteasome inhibitor MG132, fully blocked elacestrant depletion of ER, similar to the effect of MG132 treatment in preventing fulvestrant-mediated ER turnover. The combination of elacestrant with CDK4/6 inhibitors, palbociclib or abemaciclib, demonstrated additive compared with monotherapy. In addition, elacestrant inhibited growth of palbociclib-resistant MCF7 LTED cells.

Conclusion: These findings highlight the potential utility of elacestrant as a 1st or 2nd line drug in the treatment of ER+ BC. The results support the testing of elacestrant versus fulvestrant after relapse on an AI, either alone or in combination with a CDK4/6 inhibitor.
Title: Molecular features of dormancy in ER+ breast cancers

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Body: Background: Late recurrence (emergence from dormancy) is characteristic of ER+ breast cancers. Despite adjuvant endocrine therapy, many breast cancers recur decades after their initial diagnosis and treatment. Why this occurs is poorly understood.

Methods: We studied 2 independent datasets of endocrine treated, ER+ breast cancers with up to 20 years follow-up. The 1st comprised matched samples from the primary tumor pretreatment at diagnosis and the first recurrence after or during adjuvant endocrine therapy (all FFPE). The 2nd dataset comprised pretreatment biopsies only (all snap frozen). For both datasets, high quality RNA was amplified, labelled, and subjected to transcriptome analysis using the Affymetrix technology (U133 Plus 2.0). Low quality data were identified using 'simpleaffy' and 'ffpe', and removed; all tools were from the R package unless otherwise noted. Remaining data were normalized using 'frma'. Genes differentially expressed between early (≤ 3 years) and late (≥ 5 years) were selected using limma. Unsupervised hierarchical clustering and PCA explored the structure of the data.

A similar molecular analysis was done on the 2nd dataset. A classification scheme that robustly separated early from late recurrences was validated in an independent public dataset of comparable patients, array platform, and frozen tissues. We also explored features in pretreatment samples that predetermined response duration.

Results: Genes that separated pretreatment specimens by recurrence time did not separate posttreatment specimens. Specimens did not cluster in patient pairs or by site of recurrence. 8245 genes were differentially expressed between early and late recurrences in the FFPE samples, while 2400 genes were significantly different in the same comparison in the frozen samples. Initial pathway analysis was done on each dataset independently using IPA (Ingenuity® Systems, www.ingenuity.com). 70 canonical pathways were identified in common between the two datasets (pretreatment). We then looked for genes regulated in both datasets (ignores FFPE and frozen tissue as source). There were 279 genes in common that differentially regulated in the same direction (upregulated; downregulated). IPA analysis of these genes identified 49 canonical pathways. We also explored the differentially expressed gene sets using 'GSEA' (www.software.broadinstitute.org/gsea/index.jsp). Pathways consistently associated with early vs. late recurrence include integrin signaling, the unfolded protein response, endoplasmic reticulum stress, actin-based motility, and estrogen biosynthesis.

Conclusion: Analysis of pretreatment tumors can predict early recurrences from those that will remain dormant and recur much later. Recurrent tumors exhibit a remodeled molecular landscape that likely reflects the effects of treatments and/or a recreation of a niche with potentially common features at the site of recurrence. Changes in molecular signaling associated with duration of recurrence are consistent with our experimental model studies in vitro implicating UPR signaling as a major integrator of response to endocrine therapy and duration of survival. Additional data sets are being arrayed and more detailed molecular signaling studies are in progress.
**Title:** The 8p11-p12 amplicon oncogene ASH2L regulates expression of genes involved in tumorigenic processes and response to palbociclib via promoter H3K4me3

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**Body:**

**Background.** Amplification of the 8p11-p12 region, occurring in 10-30% of luminal tumors, is associated with poor prognosis and resistance to endocrine therapy. The 8p11-p12 amplicon is heterogeneous and contains several validated oncogenes, including NSD3, ZNF703, and FGFR1. ASH2L, an epigenetic regulator of chromatin, is a member of the trithorax group of proteins that promote transcription, primarily through tri-methylation of lysine 4 on histone H3 (H3K4me3) in the promoter region of target genes. The 8p11-p12 amplicon has been well-characterized previously in the SUM44 cell line and the role of overexpressed oncogenes such as NSD3 has been demonstrated. We therefore selected this cell line for further investigation of ASH2L function in the context of amplification of this oncogene, and its relationship with response to CDK inhibitors.

**Methods.** We assessed cell growth following knockdown of ASH2L with shRNA constructs compared to LacZ control in MCF7 and in the amplicon-bearing SUM44, CAMA1, and SUM52 cell lines. To assess the influence of ASH2L expression on promoter H3K4me3, we performed chromatin immunoprecipitation and high throughput sequencing (ChIP-seq) utilizing an H3K4me3 antibody in control SUM44 cells and following shRNA-mediated knockdown of ASH2L. Three biological replicates were prepared and the samples sequenced simultaneously. We treated LacZ control and ASH2L knockdown SUM44 and MCF7 cells with increasing doses of palbociclib and assessed cell growth over 7 days. From the set of genes that lost promoter H3K4me3 upon ASH2L knockdown and were downregulated in response to knockdown of ASH2L, we selected 5 genes (FBXO5, EZH2, TTK, CCNE2, and BUB1) that were downregulated in response to treatment with palbociclib according to comparative toxicogenomics database data.

**Results.** Knockdown of ASH2L reduced cell proliferation, validating that ASH2L influences cell growth regardless of amplification status. From ChIP-seq data, we found ASH2L robustly regulates H3K4me3 at the promoter region of itself and other genes of the amplicon, and genes pertaining to cell cycle and proliferation, such as EZH2 and WDR5. To determine the influence of ASH2L-regulated promoter H3K4me3 on transcription, we compared the gene set that lost promoter H3K4me3 upon ASH2L knockdown to the genes that were downregulated at the mRNA level upon knockdown of ASH2L by shRNA. This analysis identified 438 genes that were directly transcriptionally regulated by ASH2L, including cell cycle processes, palbociclib response, EZH2-regulated genes, estradiol-upregulated genes, and ESR1 interactions. SUM44 cells were more resistant to palbociclib than MCF7, and knockdown of ASH2L rendered these cells more resistant yet. Expression of FBXO5, EZH2, TTK, CCNE2, and BUB1, was reduced in the palbociclib-sensitive MCF7 cell line, however SUM44 cells did not demonstrate a reduction in transcript level for these genes after palbociclib treatment.

**Conclusion.** We identified a suite of genes with ASH2L-dependent promoter H3K4me3 that are transcriptionally downregulated upon ASH2L knockdown and discovered that these genes are predicted to be implicated in many cell cycle processes as well as response to palbociclib.
Title: Therapeutic targeting of CDK4/6 inhibitor resistant breast cancer

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Body: Background:
Selective inhibitors of CDK4/6 kinases (CDK4/6i) were recently FDA approved for use in combination with endocrine therapy (ET), and represent the new standard of care. There are however patients who do not respond or develop resistance to these treatments, and therapies are required in this setting. While there is emerging data on the mechanisms of intrinsic insensitivity to CDK4/6i as monotherapies, which include cyclin E1 amplification, CDK6 amplification and Rb deletion, there is little data on mechanisms of resistance to combined ET and CDK4/6i.

Methods:
We established MCF7 cell line and patient-derived xenograft (PDX) models that are resistant to combined ET and Palbociclib (CDK4/6i) through long-term culture, allowing us to better understand mechanisms underlying CDK4/6i resistance and to model therapeutic strategies in this setting. We also evaluated our therapeutic strategy in vitro and in vivo using MCF cell lines that are resistant to ET, and in an ER+ PDX model derived from a patient who progressed on ET.

Results:
Cells resistant to CDK4/6i alone and in combination with ET show disrupted senescent pathways, and insensitivity to the induction of senescence. MDM2 inhibitors induce cells to enter into senescence, and consequently we are investigating the use of a new generation MDM2 inhibitor (CGM097, Novartis) either in combination with CDK4/6i treatment, or following acquisition of CDK4/6i resistance to prevent exit from senescence. We evaluated a CGM097 either in combination with CDK4/6i treatment, or in combination with fulvestrant following acquisition of CDK4/6i resistance to prevent exit from senescence. CGM097 was effective alone or in combination with fulvestrant in CDK4/6i resistant cells in vitro and in vivo, and resulted in a loss of G1 cells, and a reduction in B galactosidase, a senescence marker.
Another mechanisms of CDK4/6i resistance that has been identified is CDK2 activation, which can occur through Cyclin E amplification. As a second therapeutic strategy, we screened a panel of pan-CDK inhibitors with CDK2 activity in our resistant lines, and identified that CYC065 (Cyclacel), a highly selective CDK2/9 inhibitor, had the most durable response and highest synergy with ET in long-term culture. The combined resistant models were sensitive to CYC065 in vitro and in vivo. CYC065 was mechanistically distinct to CDK4/6i's as it caused arrest in a different phase of the cell cycle and affected expression of different cell cycle proteins.

Conclusion:
An underlying mechanism of combined ET and CDK4/6i resistance is senescent escape, which allows for normal proliferation upon removal of the drug. Using our in vitro and in vivo models of combined ET and CDK4/6i resistance, we have identified two novel therapeutic strategies for this disease, which represents the next clinical challenge in ER+ breast cancer as the natural history of disease is changed with the increasing use of CDK4/6i.
Title: Androstenedione initiates rapid non-genomic signalling mediated by cytoplasmic androgen receptor in aromatase inhibitor resistant breast cancer

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Body: Aromatase inhibitors (AI) are the recommended first line therapy used to treat postmenopausal breast cancer. These compounds work by inhibiting the aromatase enzyme thus preventing the conversion of circulating androgens to estrogen; as a consequence they alter the tumour intracrinology and create an unopposed highly androgenic steroid environment. We have previously reported androgen receptor (AR) protein levels to be up-regulated in an AI resistant cell line (LetR cells), and subsequently identified a mechanism by which AR drives a more aggressive phenotype in AI resistant breast cancer [1]. In this current study, LetR cells are shown to be responsive to the weak androgen androstenedione (4AD), and treatment with this steroid drives an invasive phenotype in vitro. In support of this data, clinical studies have also reported increases in the serum levels of 4AD in patients that recur on AI therapy [2].

In the canonical pathway, androgens bind to the AR which results in a conformational change to an active state. Non-canonical AR activation occurs when ligand-transformed AR interacts with molecular partners within the cytosol to induce rapid intracellular signalling cascades. These events do not depend upon AR mediated gene transcription and occur extremely quickly within a manner of minutes [3]. In vitro studies using western blot analysis and co-localisation experiments have indicated 4AD treatment potentiates a resistant phenotype through non-genomic AR actions initiated by rapid second messenger signalling within the cytoplasm. Mass spectrometry (LC–MS/MS) analysis has identified androgen-mediated, rapid cytoplasmic AR protein interactions, resulting in the identification of AR partners unique to our resistant model. Of note, evaluation of AR protein and p-ERK1/2 in a cohort of primary breast cancer patients (n=363) demonstrated that high levels of cytoplasmic AR significantly diminished survival in ER+ PR- patients (p=0.023, Fisher’s exact). Elevated pERK1/2 when concomitant with increased levels of cytoplasmic AR result in a significant decrease in the period of disease free survival (p=0.018). Further investigations into these AR interactors will help elucidate mechanisms of resistance to AI therapy, and in turn these novel AR protein partners will aid the identification of patients who would benefit from anti-AR therapy.

References
Title: Anti-tumor activity of elacestrant (RAD1901) in combination with alpelisib (BYL-719) in patient-derived xenograft models of ER+ breast cancer

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Body: Estrogen-receptor positive (ER+) breast cancers make up approximately 70% of all breast cancers diagnosed. While patients with ER+ breast cancer have a better prognosis than other subtypes of breast cancer, the majority of those with advanced metastatic disease will eventually relapse. This has been attributed, in part, to mutations in the ER gene that result in constitutive activation of ER and contribute to aromatase inhibitor treatment resistance. As a strategy to deliver a more durable response in this setting, the use of selective estrogen receptor degraders (SERDs) that target and inhibit both wild-type and mutant ER has gained widespread attention. Indeed, fulvestrant, the only approved SERD on the market, is currently used as a second-line therapy in the metastatic setting, however, the intramuscular route of administration and pharmacokinetic properties of fulvestrant have fueled the development of orally bioavailable SERDs. We have previously described elacestrant (RAD1901), a novel and orally bioavailable selective estrogen receptor degrader (SERD) as an inhibitor of ER+ breast cancer growth in preclinical models, including those that harbor ER mutations and those that are insensitive to fulvestrant. In addition to ER mutations, the activation of parallel oncogenic pathways can also drive endocrine resistance, with the PI3K/Akt/mTOR pathway chief among those driving growth and treatment resistance to endocrine therapy. Consistent with these above-mentioned findings, recent clinical strategies to treat advanced ER+ disease have involved combining SERDs with PI3K inhibitors. Alpelisib (BYL-719) is a PI3K-alpha specific inhibitor that is being developed in combination with endocrine agents for the treatment of ER+ breast cancer. Here, we examined the effect of elacestrant in combination with alpelisib, in two ER+ breast cancer PDX models (one harboring wild-type ER and one harboring a Y537S mutation in the ER gene). The combination of elacestrant (10mg/kg) and alpelisib (35mg/kg) was well tolerated and resulted in significant tumor growth inhibition in both PDX models. Interestingly, in the mutant ER PDX model, the combination resulted in significantly greater growth inhibition relative to either compound alone. These data suggest the dual inhibition of the ER and PI3K signaling pathways with elacestrant and alpelisib produces significant anti-tumor activity in clinically-relevant PDX models, including those harboring ER mutations.
Body: Introduction: Approximately 25-30% of breast cancers overexpress human epidermal growth factor receptor 2 (HER2). HER2-positive breast cancers are more likely to be diagnosed in younger women and are highly aggressive. Currently, there are few treatments options available specifically for HER2-positive breast cancer which includes Herceptin, Kadcyla, Perjeta and Tykerb. There is a higher risk of recurrence of HER2-positive breast cancers compared to HER2-negative breast cancers. Hence it is important to devise effective strategies to prevent/treat HER2-positive breast cancer. We had earlier shown that short-term treatment with pregnancy levels of estrogen plus or minus progesterone was highly effective in inhibiting mammary tumor growth in MMTV. Here we attempt to understand the mechanism behind this protection.

Materials and Methods: MMTV-neu transgenic mice were treated for 3 weeks, starting at 7 weeks of age, with 100 µg of estradiol in Silastic capsules. This dose of estradiol used in the Silastic capsules results in pregnancy levels of estradiol in the circulation. The control animals received empty Silastic capsules for the same duration. Mice were palpated weekly for 9 months to monitor for mammary cancer development. A set of mice were sacrificed at 7, 10, 16, 22, 28 and 36 weeks of age. Mammary gland was surgically isolated. Inguinal mammary glands were prepared as wholemounts. A piece of the mammary gland was fixed in formalin for histopathology and the rest was snap frozen in liquid nitrogen and stored at -80°C for further molecular analysis. Pathway focused microarrays (Cancer Pathway Finder, and PI3K-AKT Signaling) were performed and the differential regulated genes were validated using real-time RTPCR and immunoblotting. All data obtained were statistically analyzed using Graphpad Prism version 5.03. The repeated measures analysis of variance was used to evaluate the dose and time response to hormone treatment. Multiple comparisons between groups with significant differences were analyzed using Dunnett post–hoc test. Paired t-test was done to analyze intergroup differences. The values of p <0.05 were considered as statistically significant.

Results: Short-term treatment with pregnancy levels of estradiol significantly reduced the mammary cancer incidence and multiplicity, while it significantly increased mammary cancer latency. Pathway focused microarray analysis of mammary glands at different time points demonstrated that mice treated with short-term estradiol has persistent changes in gene expression involved in key signaling pathways involved in cancer. Pro-angiogenic genes (Angpt1, Fgf2, Vegfc), anti-apoptotic genes (Bcl2, Birc3), pro-proliferative/survival genes (Akt, Ccnd1, Igf1r, Mtor, Pi3k) were significantly down-regulated in the mammary gland of short-term estradiol treated mice. On contrary, pro-apoptotic genes (Bad, Casp, Cflar) and anti-proliferative/survival genes (Pten, p53) were up-regulated.

Conclusion: Our data demonstrates that short-term treatment with pregnancy levels of estradiol is highly effective in inhibiting mammary carcinogenesis in MMTV-neu mice. This protection against HER2 positive mammary cancers is achieved by altering key signaling pathways involved in cancer growth and progression.
Title: Long non-coding RNA DSCAM-AS1 regulates G1/S cell cycle transition and is an independent poor prognosis factor in luminal breast cancer with endocrine therapy

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Body: While an increasing number of long non-coding RNAs (lncRNAs) have been revealed to play fundamental roles in tumorigenesis and progression of triple negative breast cancer (TNBC), lncRNA studies on luminal breast cancer, which accounting for two thirds of all breast tumor, are still rare. DSCAM-AS1 is the first and only intensively studied lncRNA with high specific expression in luminal breast cancer, directly regulated by estrogen receptor α (ERα), and playing vital roles in tumor proliferation, invasion and tamoxifen resistance. However, the detailed function of DSCAM-AS1 in tumor progression and its clinical significance are still not clear.

In this study, we revealed that DSCAM-AS1 regulates cell proliferation and colony formation by inducing cell cycle G1/S transition. RNA-seq analysis demonstrated that DSCAM-AS1 participates in crucial biological processions including DNA replication, cell cycle G1/S phase transition, sister chromatid cohesion, chromosome segregation, protein localization to chromosome and DNA recombination.

For clinical analysis, a total of 399 luminal breast cancer patients were enrolled, with median follow-up time of 58.91 months (Interquartiles range, 42.90-70.65) and 72 events. The median age was 48 years (Interquartiles range, 42-58). All the patients have received tumor resection. 165 (41.35%) patients have received radiotherapy and 342 (85.71%) patients were treated with chemotherapy. With a cut-off value of 13.05 (ΔCT value), DSCAM-AS1 were high expressed in 239 patients, and low expressed in 160 patients. In the total cohort, univariate survival analysis revealed that characteristics including tumor size (p=0.005), grade (p=0.004), positive lymph nodes number (p<0.0001) and ki-67 (p=0.012) were correlated with prognosis. High expression of DSCAM-AS1 was inclined to shorter disease free survival (DFS), although no statistic difference has been reached (p=0.061). DSCAM-AS1 was not an independent factor for the total cohort (HR, 1.46; 95% CI, 0.86-2.46; p=0.16).

Of all the 399 patients, 309 (77.44%) have received more than one year endocrine therapy and were included into the endocrine therapy group. In the endocrine therapy group, patients with high DSCAM-AS1 expression level had shorter DFS in the univariate analysis. In the multivariate analysis, DSCAM-AS1 (HR, 2.22; 95%CI, 1.16-4.23; p=0.016) together with grade (HR, 1.88; 95%CI, 1.11-3.21; p=0.02) and positive lymph nodes number (HR, 2.04; 95%CI, 1.47-2.84; p<0.0001) were independent prognosis factors.

In conclusion, DSCAM-AS1 is a prognostic factor, although no statistical significance, in patients with luminal breast cancer. In luminal breast cancer patients with endocrine therapy, high expression of DSCAM-AS1 acts as a poor independent factor to affect patients' DFS. DSCAM-AS1 is a promising clinical therapy target to prolong luminal breast cancer patients' survival, together with endocrine therapy.
Title: Elacestrant (RAD1901) demonstrates anti-tumor activity in a fulvestrant-resistant PDX model

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Body: Background: Breast cancer is subdivided into categories based on tumor receptor status, with estrogen-receptor positive (ER+) breast cancer making up approximately two-thirds of all breast cancers diagnosed. In advanced or metastatic disease, dependence on ER signaling is often retained even after multiple rounds of endocrine therapy, supporting the use of ER targeting agents for several lines of treatment prior to chemotherapy. Selective estrogen receptor degraders (SERDs) have gained recent attention as ER antagonists given their ability to target and degrade ER. Fulvestrant, the only approved SERD on the market, is currently used as a second-line therapy in the metastatic setting, however, the intramuscular route of administration and pharmacokinetic properties of fulvestrant have fueled the development of more potent and orally bioavailable SERDs. We have previously described elacestrant (RAD1901), a novel and orally bioavailable SERD, as an inhibitor of ER+ breast cancer growth in preclinical patient-derived xenograft (PDX) models, including those that are insensitive to fulvestrant. Here, we describe elacestrant activity in an ER+ PDX model that had been treated with multiple rounds of fulvestrant treatment.

Methods: An ER+/PR+ PDX model, MAXF-713, derived from a treatment-naïve patient, was passaged multiple times in the presence of fulvestrant over the course of a year. After each round of fulvestrant treatment, elacestrant or fulvestrant were evaluated for anti-tumor activity. Pharmacodynamic endpoints including changes in ER and ER target gene expression were also evaluated.

Results: Despite repeated exposure to fulvestrant, the PDX model retained sensitivity to elacestrant. In fact, regardless of the number of prior rounds of fulvestrant treatment, elacestrant produced consistent tumor growth inhibition within every passage, while the effects produced by fulvestrant exhibited a high degree of variability.

Conclusions: Our data demonstrate that elacestrant is a SERD that can inhibit tumor growth in a fulvestrant-resistant preclinical setting and provides rationale for examining elacestrant in the clinical setting in patients that have progressed on fulvestrant treatment.
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Title: Estrogen receptor α-dependent transcriptional induction of selenium-binding protein 1 increases the sensitivity of tamoxifen treatment in breast cancer

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Body: Reduction of selenium-binding protein 1 (SELENBP1) has been frequently observed in various solid tumors, and associated with poor clinical prognosis, implicating SELENBP1 as a potential tumor suppressor. However, the molecular mechanism underlying the transcriptional regulation of SELENBP1 remains poorly understood. In this study, we first analyzed the relationship between SELENBP1 mRNA levels and clinical or pathological features of breast cancer in METABRIC datasets, and found that SELENBP1 mRNA levels were differentially expressed in PAM50 molecular subtypes with highest levels in Luminal A/B subtype and lowest levels in Basal-like/Claudin-low subtype, indicating that SELENBP1 might function as the downstream player of estrogen receptor α (ERα). Following the adjustment of clinicopathological characteristics including the menopausal status, ER/HR/ERBB2 status, TNM stage and histological grade, the Kaplan-Meier survival analysis surprisingly showed that patients with higher levels of SELENBP1 mRNA (n=43) had shorter survival time than those with moderate levels of SELENBP1 expression (n=102) in Luminal A subtype (Hazard Ratio 1.59; 95% CI 0.99-2.54; Logrank p=0.0367), although the patients with lower SELENBP1 levels were consistent the poor prognosis in Basal-like/Claudin-low subtype (Hazard Ratio 1.54; 95% CI 0.97-2.44; Logrank p=0.0356). To elucidate this controversy, we knocked down the expression of ERα in MCF-7 cells using shRNA targeting ERα, and found that knockdown of ERα resulted in the down-regulation of mRNA and protein expression of SELENBP1. In addition, SELENBP1 promoter-driven luciferase reporter assay reveled ERα regulated the expression of SELENBP1 at transcriptional level. Interestingly, we further found that the addition of 4-hydroxytamoxifen (4-OHT) led to a dramatic reduction of SELENBP1 protein expression in MCF-7 cells, while forced expression of SELENBP1 in MCF-7 cells significantly enhanced 4-OHT-mediated inhibition of anchor-independent cancer cell growth. Collectively, these results suggested that the transcriptional induction of SELENBP1 by ERα might function as a downstream tumor suppressor in breast cancer cells, but endocrine therapy led to the down-regulation of SELENBP1 expression due to the blocking of ERα signaling in the patients with Luminal subtype, which was likely to explain the observation of differential predictive value for SELENBP1 mRNA levels in the Luminal and Basal-like/Claudin-low subtypes. Further investigation in vitro and in vivo has been ongoing in our laboratory, and the results from which will help us better understand the clinical relevance for SELENBP1 as a promising therapeutic target in improving the efficacy of endocrine therapy.

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Endocrine medical treatment in breast cancer, is that enough?

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Body: Endocrine treatment has been in the management of hormone receptor breast cancer for a few decades, but never as the first line of treatment. Now in the biomolecular era, we are able to select patients where endocrine treatment can be considered the first line of treatment.

Methods: During the months of May 2011 until June 2017, patients with ductal/lobular, in-situ and invasive cancer were treated medically with endocrine treatment as first choice of treatment (Tamoxifen/AI), due to medical morbidity or as requested by patients. The use of biomolecular assays helped to support the treatment decision. The infrastructure of ABC as a comprehensive center with all the modalities for diagnostic and interventional breast imaging center allow us to do all the follow up with the same health professionals. The assessments of the follow-ups were based on clinical, imaging (MRI, ultrasound and mammogram), and pathological responses.

We use the term "complete clinical response" on patients where the clinical history and the physical examination demonstrate complete improvement of the breast cancer. "Complete imaging response" is referred to those patients that before treatment had a breast MRI with enhancement lesions and in the follow-ups show no more enhancements. "Complete pathological response" refers to patients which had no residual cancer on the surgical specimen.

Results: We followed a total of 32 patients for an average period of 20.12 months (range=2-63 months), and an average age of 69.69 (range=45-86). Twenty-six of these patients (81.2 %) had Invasive Ductal Carcinoma, 6 patients (18.7%) had DCIS, 23 of the patients (71.88%), were only treated with endocrine treatment. Nine patients (28.13%) were treated with endocrine therapy plus surgery. The decision for surgery was a personal request of the patient, not a failure of the endocrine treatment.

The 32 patients (100%) had a complete clinical response. Twenty-five patients (78.13%) presented a complete imaging response. From the patients who had surgery, 1 patient (11.11%) presented a complete pathological response, 6 patients (66.67%) presented down staging of the tumor, and 2 patients (22.22%) presented no change.

Twenty-nine of the 32 patients (90.63%) were able to obtain the recurrence score by Twenty-one Genes. Twenty-four of those had a low recurrence score, 4 had an intermediate score, and 1 patient had a high recurrence score.

Discussion: Even though this is a small group of patients, there was no failure of treatment, mortality or progress of the disease. It demonstrated that, on selective patients, the effectiveness of endocrine treatment is possible, and can be used as the first line treatment. The selection of those patients was supported with the use of the 21 genes.

Conclusion: Nowadays endocrine treatment, based on clinical and biomolecular assays, can be considered safe to use as first line of treatment for invasive and non-invasive breast cancer for those patients that refuse surgery or chemotherapy.
G protein-coupled estrogen receptor expression in lymph node metastasis and contralateral breast cancer during endocrine treatment

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Background: G protein-coupled estrogen receptor (GPER) is a putative estrogen receptor (ER) with potential clinical implications in breast cancer. Depending on subcellular location, cut-off and treatment, GPER has been proposed as both a marker of aggressive disease and of good prognosis. The correlation with known prognostic factors appears to be biphasic. Furthermore, plasma membrane GPER-overexpression (GPER\textsubscript{PM}) is associated to a worse prognosis, suggesting potential receptor mutation or amplification. In vitro studies also show that tamoxifen upregulates GPER in breast cancer cells, suggesting GPER as a mediator of endocrine resistance. To further clarify the role of GPER in breast cancer and endocrine treatment we studied GPER during evolution from primary tumor to lymph node metastasis (lgl-met), and from first tumor (BC1) to a second contralateral tumor (CBC), with/without endocrine treatment (mainly tamoxifen) in between. As a CBC developed despite adjuvant treatment given for BC1 is presumably resistant to this treatment, it may be used to study endocrine treatment escape mechanisms \textit{in vivo}.

Patients and methods: From a well-defined population-based cohort of CBC-patients we constructed a unique tissue-microarray including 688 patients with metachronous CBC. Overall GPER staining intensity (0-5) (GPER\textsubscript{overall}) and GPER\textsubscript{PM} (present/absent) was evaluated by two investigators in BC1 (n=559), CBC (n=595) and corresponding lgl-met (n\textsubscript{BC1}=151 and n\textsubscript{CBC}=158). Impact on survival was analyzed using the Kaplan-Meier method and Cox regression, and p-values calculated with the log rank test. Association was tested with a chi-square test (for trend when appropriate), and differences between paired samples with Wilcoxon signed rank test.

Results: GPR30\textsubscript{overall} showed the expected biphasic association with ER-\alpha and the progesterone receptor (PgR) in BC1 and CBC, where high and low GPER expression associated with ER/PgR negativity (p<0.001). High GPER expression was also associated with increased Ki67 expression (p=0.04 and p=0.08). Similar results were seen for GPER\textsubscript{PM} (ER/PgR-negativity p<0.001, high Ki67 p<0.001). There was no association between GPER in BC1 and CBC, and prior endocrine treatment did not seem to affect GPER expression in CBC. However, a significant association between primary tumor and corresponding lgl-met was seen in both BC1 and CBC. Further, a comparison between BC1/CBC and corresponding lgl-met showed a decrease in GPER\textsubscript{overall} (p<0.001), but an increase in GPER\textsubscript{PM} (p=0.01). A high CBC GPER expression was associated with a worse OS, both when considering GPER\textsubscript{overall} (p=0.02, HR=1.18, 95%CI: 1.05-1.32) and GPER\textsubscript{PM} (p=0.007, HR=1.61 95%CI: 1.14-2.29). Similar results were seen when analyzing survival in relation to CBC lgl-met GPER expression.

Conclusion: A high GPER expression in CBC and matched lgl-met is associated with worse OS. The overall expression of GPER decreases from primary tumor to lgl-met, but plasma membrane staining increases, supporting a mutation or amplification of the receptor. In addition, GPER expression correlates with other markers of aggressive disease, highlighting the potential as a novel treatment target. However, prior tamoxifen treatment did not alter tumor GPER expression.
Title: Differential regulation of ER protein-turnover in invasive lobular carcinoma cells

Body: Background: Invasive lobular breast carcinoma (ILC) accounts for 10-15% of breast cancers diagnosed annually. ILCs are more likely to be positive (90-95%) for ER compared to IDC (60-70%), and there is some evidence that endocrine treatment response might be different in patients with IDC vs ILC. We asked the question whether there were differences in ER protein steady state levels, and/or turn-over rates.

Methods: We utilized TCGA dataset to compare ESR1 mRNA and ER protein levels between ER+ ILC (n=137) and IDC (n=554). ER H-scores and ESR1 mRNA levels were analyzed from patients with ER+ ILC (n=143) and IDC (n=877) seen at UPMC Magee Women's Hospital. Correlation analysis with Pearson's (r) and Spearman's rank order coefficient (ρ) was used to study the relationship between mRNA and protein levels. Basal and ligand induced ESR1 mRNA and ER protein expression and turn-over were determined in a panel of estrogen responsive ER+ IDC (MCF-7, T47D and ZR75-1) and ILC (BCK-4, MDA-MB-134 VI (MM134), SUM44PE) cell lines to identify potential mechanisms that can contribute to differential expression of ERα protein.

Results: TCGA database analysis revealed significantly lower ESR1 mRNA and ER protein levels in ER+ ILC compared to IDC tumors. Analysis of data from our Magee hospital showed similar ER IHC H-scores for ER+ ILCs and IDCs despite having significantly lower ESR1 mRNA in ILC. In both the study sets, the correlation between ER mRNA and protein levels were found to be significantly weaker in ER+ ILC than IDC suggesting subtype specific increased synthesis and/or stability of the receptor protein. ILC cell lines MM134 and SUM44 have increased levels of ER protein compared to IDC cell lines. Estradiol decreased the levels and half-life of ER protein in all IDC cell lines tested. In MM134 and SUM44PE ILC cell lines, estradiol decreased the rate of degradation of ER and increased its half-life. In MM134 cells, treatment with estradiol induced a dose- and time-dependent increase in ER, which is associated with a sustained level of elevated phosphorylation at Ser 118.

Conclusions and ongoing/future studies: The estradiol-induced ER protein stability in a subset of ILC cell lines suggest a possible mechanism leading to weaker ER mRNA-protein correlation in ILC tumors. We currently do not know if and how this observation might be linked to antiestrogen response in ILC. We have recently initiated a TBCRC-supported trial in which we will compare the effect of different endocrine therapies in patients with ILC. Access to pre and post therapy samples will provide the opportunity to study ER levels and its downstream signaling as a function of antiestrogen treatment.
**Title:** Heat shock protein 27 (HSP27) and HER2 positively correlate in breast cancer and effect cell responsiveness to neratinib and cMET inhibitor

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**Body:** Background: Upregulation of heat shock protein-27 (HSP27), a key member of the HSP family, has been shown to confer resistance to chemotherapy and radiotherapy in solid tumours including breast cancer. Evidence is also emerging that HSP27 is linked to the metastatic spread of breast cancer and key cellular traits including cellular migration. Neratinib is an orally available tyrosine kinase inhibitor that irreversibly binds and inhibits EGFR, HER2 and HER4 receptor tyrosine kinases. cMET, the receptor for hepatocyte growth factor and target for cancer therapies, has been shown to be trans-phosphorylated by EGFR. In the current study, we evaluated HSP27 expression within a breast cancer cohort and its implications in cellular responsiveness to cMET inhibition and neratinib.

Methods: HSP27 transcript expression was analysed in our chemotherapy naïve breast cancer cohort (n=124) using quantitative PCR (qPCR) and compared to clinic-pathological information including long-term survival over a ten year follow up period. In addition, the correlation between HSP27 and HER2 expression was explored using Spearman Rank order. High-throughput cell migration analysis was performed using ECIS, on MCF-7 control and HSP27 siRNA knockdown cells in conjunction with neratinib and PHA 665752, a small molecule cMET inhibitor.

Results: In our primary breast cancer cohort, there were no significant associations between HSP27 transcript expression levels and tumour grade, TNM or estrogen receptor (ER) status. Combined survival expression analysis indicated that the worst patient prognosis was associated with high levels of both HER2 and HSP27 and high HER2 and low HSP27 whereas best patient prognosis was associated with low HER2 and low HSP27 expression. Knockdown of HSP27 in MCF7 cells brought about a reduction in cellular migration compared to the control. Additionally, this reduction was enhanced by the addition of neratinib, in a concentration dependent manner, and also cMET inhibition when individually treated. Furthermore, the greatest inhibitory effects on MCF-7 migration were seen following HSP27 knockdown and combined treatment with neratinib and PHA 665752.

Conclusions: Our current data suggests that HSP27 confers low sensitivity to drugs such as neratinib and PHA 665752, particularly in relation to cellular migration and hence potentially metastasis. Therefore, the targeting of HSP27, HER2 and cMET appear to act synergetically to regulate cellular migration in vitro. Furthermore, clinically expression of HER2 and HSP27 may serve as a prognostic marker for breast cancer survival. Hence, combination therapies that target both HSP and HER2 pathways may provide new clinical opportunities for preventing breast cancer progression.
Title: Provider practice and compliance with NCCN clinical guidelines for BRCA1/2 testing: Findings from Dr. Susan Love Research Foundation's The Health of Women (HOW) Study™

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Body: BRCA1/2-attributable cancers disproportionately contribute to breast cancer mortality, but are relatively infrequent in the general population, making healthcare practices surrounding BRCA1/2 mutation carrier status difficult to study. This analysis leverages a large dataset of over 40,000 individuals with and without breast cancer from Dr. Susan Love Research Foundation's The Health of Women (HOW) Study™ to evaluate factors associated with healthcare provider compliance with National Comprehensive Cancer Network (NCCN) guidelines for BRCA1/2 testing. Data from women ages 18 and older who completed baseline and family history questionnaires were included in our sample (n=22,410). We examined whether: (1) genetic testing receipt was aligned with NCCN guidelines for BRCA1/2 genetic testing, (2) guideline-consistent use of BRCA1/2 testing varied by race and education, (3) those who had genetic testing received pre-test genetic counseling, and (4) guideline-consistent BRCA1/2 testing differed among those who received pre-test genetic counseling. In this population, 4326 women (19.5%) reported BRCA1/2 testing. Of these women, 70.3% of women were eligible for testing based on NCCN guidelines (vs. 29.8% who did not qualify). Women with higher levels of education were less likely to be over-tested or under-tested than those with lower levels of education (p<0.001); no differences were observed by Hispanic ethnicity (Hispanic vs. non-Hispanic, p=.71) or race (white vs. non-white, p=0.23). Among those tested for BRCA1/2, 65.6% (n=2976) received pre-test genetic counseling. Those who received pretest counseling were more likely to receive genetic testing consistent with NCCN guidelines than those who did not receive pretest counseling (73.8% vs 63.6%, p<0.001). 58% received post-test counseling by a genetics provider (n=2515) compared to 33.9% who received genetic results from their primary care provider or another provider (n=1465). Those who received genetic test results from a genetics provider were more likely to have received BRCA1/2 testing that was compliant with NCCN guidelines than those who received their genetic test results from another type of provider, though by a modest amount (74.2% vs 70.6%, p=0.01). In this population, we found that pretest-genetic counseling was associated with higher rates of NCCN guideline-consistent recommendations for BRCA1/2 genetic testing, and a woman's educational level appears to be associated with higher rates of guideline concordant use of BRCA1/2 testing. Overall, these findings showed higher rates of BRCA1/2 testing and receipt of counseling than previously reported, which may be partially reflective of this unique study population of motivated volunteers. As genetics is increasingly incorporated into cancer prevention, there is an urgent need to examine adherence to genetic testing guidelines to ensure appropriate use.
Title: Germline analysis of breast cancer patients with abnormal somatic results: Ancillary assessment or critical co-diagnostic?

Body: Background: Tumor genetic testing (TGT) is increasingly used for planning cancer treatment and identifying appropriate clinical trials. Emerging literature shows that 4–12% of genetic variants identified on TGT are also present in the germline, conferring hereditary cancer risk. Germline genetic testing (GGT) guidelines were recently expanded to include the identification of a BRCA1/BRCA2 variant on TGT as an indication for germline analysis. We evaluated the diagnostic yield of current GGT guidelines by assessing the rate of pathogenic and likely pathogenic (P/LP) germline findings in a series of patients who had a variant identified on TGT and underwent GGT. Methods: We analyzed de-identified data from 185 sequential patients with various tumor types who had TGT and GGT. Personal and family histories were compared to all available NCCN guidelines for GGT. Results: Sixty-four of 185 patients (34.6%) had at least one P/LP germline variant, and among these patients, 42% (27/64) had variants in BRCA1/BRCA2. Variants in all but one patient (26/27) were also found on TGT. Fourteen of 27 (52%) patients had a personal diagnosis of cancer not typically associated with BRCA1/BRCA2, including colorectal (5), lung (3), and one each of cervical, cholangiocarcinoma, gastric, thymus, thyroid, and uterine. Furthermore, prior TGT results were the only reason GGT guidelines were met in 12 of 27 (44%) patients with germline BRCA1/BRCA2 variants. Among 34 patients with a personal history of breast or ovarian cancer, a P/LP germline variant was identified in nine (26%); the majority (5 of 9) were in non-BRCA1/BRCA2 genes including CDKN2A (1), FANCA (1), MUTYH (1), and PALB2 (2). Notably, the patient with the CDKN2A variant did not meet current breast cancer guidelines for GGT, and one patient with breast cancer and a germline BRCA2 mutation only met GGT guidelines due to prior TGT results. Discussion: Genetic testing guidelines have begun to reflect the opportunity for TGT to identify families at risk for hereditary cancer. Expanding GGT criteria to include TGT results is critical for capturing patients who may not otherwise receive GGT. Our data showed a substantial diagnostic yield in patients—including those with breast or ovarian cancer—who completed GGT after variant identification on TGT. Although current genetic testing guidelines capture the portion of these patients with a BRCA1/BRCA2 mutation identified with TGT, our data suggest that P/LP variants in other genes should also be considered during the evaluation of TGT results for subsequent GTG. Finally, the broad spectrum of tumor types with BRCA1/BRCA2 P/LP variants emphasizes the need for all clinicians, regardless of subspecialty, to be aware of current GTG recommendations when TGT identifies a BRCA1/BRCA2 variant and the potential implications of GTG, including targeted therapy, screening, prevention, and family testing.
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Title: Characteristics of CHEK2 mutation carriers in a large academic health center in Michigan

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Body: Background: Germline mutations in CHEK2, a cell-cycle checkpoint kinase, are associated with increased susceptibility to breast, colon, and other cancers. The clinical characteristics and cancer risks of patients with CHEK2 mutations is under investigation.

Methods: Patients identified with a pathogenic germline CHEK2 mutation between September 2013 and April 2017 were evaluated. Clinical multigene panel testing using next generation sequencing technologies was utilized. All patients received comprehensive pre- and post-test genetic counseling. Genetic testing results, demographics, tumor characteristics and outcomes were analyzed.

Results: A total of 107 CHEK2 mutation carriers were identified, of whom 88 (82%) were females. The vast majority were Caucasian (99%), and of those, 17 (16%) had Ashkenazi Jewish ancestry. The median age at time of genetic testing was 52 (range: 22-89). The most common mutations identified were: I157T (28%), c.1100delC (27%), p.S428F (15%) and c.1427C>T (9%). Seven of these patients (7%) were found to carry a second pathogenic cancer risk mutation: BRCA, ATM, NBN, NF1, and MUTYH. One patient was found to carry 3 pathogenic mutations (1100delC, ATM, and BRCA). Sixty-three (59%) patients had a prior diagnosis of malignancy, with a mean age of diagnosis of 53. Of the 88 females, the most common type of malignancy was breast cancer (55%), with a mean age of diagnosis of 52 (range: 35-79). Of the 19 males, breast cancer was seen in 4 patients. The most common known histopathology was invasive ductal carcinoma (72%), followed by DCIS (15%), invasive lobular carcinoma (9%), and papillary carcinoma (4%). Majority of the patients had breast tumors with low or moderate grade (62%), less than two centimeters (61%), node negative (70%), and estrogen/progesterone receptor positive/HER2neu negative (96%). Two patients had triple negative breast cancers. Of the 48 female mutation carriers with breast cancer, 38% underwent bilateral mastectomy. The 1- and 5-year survival was 100% with a median follow up of 57 months. Five of the 48 females developed a contralateral breast cancer, with a median time to contralateral recurrence of 6 years (range: 3-17). Two patients developed in-breast tumor recurrence at 9 and 19 years, respectively. Other cancers observed were papillary thyroid cancer (3 patients), melanoma (2 patients), and prostate (2 patients). One patient developed angiosarcoma of the chest wall two years after radiation therapy.

Conclusion: Our study describes the unique clinical characteristics of CHEK2 mutation carriers in a US-based clinic at Beaumont Health. Majority of breast cancers were early stage, hormone receptor positive and demonstrated excellent outcomes. Despite the early stage, a significant proportion of patients underwent bilateral mastectomy. Additional pathogenic mutations were identified in 10% of patients; validating the importance of panel testing in assessing cancer risk. Future studies are needed to better define the clinical presentation, cancer risks, mutational spectrum, and outcomes of CHEK2 mutation carriers in order to provide tailored screening and management guidelines for this emerging population.
Title: Germline alterations in African-American versus Caucasian patients with triple-negative breast cancer in the era of multi-gene panel testing

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Body: Background. Triple negative breast cancer (TNBC) has been associated with a relatively high rate of germline BRCA1/2 deleterious mutations (11-20%). With use of panel testing, additional predisposition genes are being identified. Among Caucasian (CC) patients (pts), pathogenic germline variants in BRCA1/2 are mainly associated with TNBC. However, these may not fully explain the higher incidence of TNBC among African Americans (AA). Additionally, partly due to under-testing among AA, the distribution of predisposition genes for AA TNBC pts is unknown, and a greater proportion of germline alterations may be classified as a variant of uncertain significance (VUS). We hypothesized that additional clinically significant germline mutations in genes, other than in BRCA1/2, may explain the increased incidence of TNBC among AA, which may be better elucidated by panel testing.

Methods. A retrospective chart review was completed of all self-identified CC and AA TNBC pts who presented to two institutions from 10/2013 to 12/2016. A total of 596 pts were analyzed. 434 (73%) were CC and 162 (27%) were AA. Clinicopathologic features including histologic subtype, age, gender, bilateral disease and stage were examined. Patients were assessed for meeting the National Comprehensive Cancer Network (NCCN) criteria for genetic testing in TNBC, type of testing performed, and the results. The distribution of mutations in higher penetrance genes (BRCA1, BRCA2, and PALB2), pathogenic variants in other genes, and VUS in AA vs. CC were analyzed. Significance was determined using a two-tailed Fisher exact test with significance of p<0.05.

Results. 306/434 (71%) CC and 98/162 (60%) AA TNBC pts met criteria for genetic testing (p=0.023). For those who met NCCN criteria for testing, 216/306 (71%) CC and 66/98 (67%) AA TNBC pts underwent testing (p=0.61). For all pts, the average age of first diagnosis was 59.5 (SD 14) for CC vs. 62.4 (SD 12.9) for AA pts. For pts who underwent testing, regardless of meeting NCCN criteria, 18% (40/221) of CC vs. 12% (8/68) of AA patients had a mutation in a higher penetrance gene (p=0.27), 1% (3/221) of CC vs. 3% (2/68) of AA had a mutation in other genes (BRIP1, Lynch genes; p=0.34), and 16% (36/221) of CC vs. 18% (12/68) of AA had a VUS (p=0.982). Of VUS, 42% (5/12) in AA vs. 19% (7/36) in CC were in higher penetrance breast cancer genes (p=0.14).

Conclusion. More CC than AA TNBC pts met criteria for genetic testing. This difference may in part be explained by the later age at diagnosis for AA given that age is a major factor in determining genetic testing. Broadening eligibility for testing in AA may help to identify more patients with an underlying genetic predisposition to TNBC. No difference was seen in the frequency of higher penetrance genes, other genes, and VUS between AA and CC pts. However, important non-BRCA genes were identified with panel testing in both AA and CC pts. In addition to broadening testing criteria for existing multi-gene panels, further genetic analysis may be necessary to explain the predisposition to TNBC in AA pts. To our knowledge, this is the first report of evaluation of predisposition genes among AA TNBC pts using germline panel testing.
Body: Background:
Breast specialty physicians play an active role in cancer genetic risk assessment and testing. Traditionally, NCCN guidelines have been the primary reference for patient selection. Although emerging research indicates that the rate of pathogenic mutations is higher than originally suspected in the general population, few studies have examined the broad use of genetic testing in patients who present to breast practices: those referred for risk assessment because of perceived higher risk or who have a diagnosis of breast cancer.

Methods:
An IRB-approved multicenter prospective data collection was performed that included 13 community-based breast physicians experienced in cancer genetic risk assessment and testing. Consecutive patients were identified as test candidates based on perceived and actual risks for hereditary breast cancer. A single test price was used to eliminate cost as a variable in panel selection. A total of 226 patients were tested and demographic data collected. Physicians reported whether patients met NCCN guidelines or not. Patients met guidelines criteria as reported by their physician 65% of the time. The majority of patients (77%) were tested for >14 genes. A recent diagnosis of breast cancer was reported for 61.5% (139/226) of patients.

Results:
Among 226 tested patients, 13.9% had a pathogenic mutation. The most common positive findings were BRCA1, BRCA2, CHEK2 and MUTYH.

Among patients who met NCCN criteria for testing, 12.2% had pathogenic mutations (50.0% of which were in BRCA1/2); whereas 11.1% of the patients who did not meet NCCN criteria had pathogenic mutations (28.6% of which were in BRCA1/2). Patients under 50 had pathogenic mutations 13.5%; patients over 50, 12.3% of the time. A higher percentage of patients younger than 50 had BRCA1/2 mutations (53.3% vs. 40.0%).

Conclusions:
Patients who did not meet NCCN genetic testing guidelines as reported by their physicians had a similar percentage of pathogenic mutations compared to patients who met guidelines.

Expanded panel testing yields more pathogenic hereditary mutations that may be actionable.

Patients grouped by age, under 50 and over 50, had different mutation distributions.

Expanded panel testing accounts for the essentially equal number of patients with pathogenic mutations who did not meet the testing criteria. Non NCCN criteria patients had a lower incidence of the most common mutations (BRCA 1/2), but still yielded actionable mutations.

These results support expanding evidence that equivalent rates of mutations may be found regardless of whether patients meet current testing criteria.

Discussion:
Although the sample size was small, the results suggest benefits to expanding testing criteria and has led to large multi-center study, currently underway, involving 20 centers, primarily community based, utilizing a single large panel test and aimed at enrolling 1000 patients. This study is focused on mutation rates in a vetted and equivalent split of NCCN/Non NCCN patient populations. Results will be available in the fall of 2017 and included in the final presentation.
Title: Frequency of mutations in multi-gene panel testing of 3,011 breast cancer patients

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Body: Objectives:
Here, we describe the characteristics of 3,011 individuals with a self-reported diagnosis of breast cancer who received a multi-gene panel genetic test for hereditary cancer risk.

Methods:
These 3,011 patients were referred physician order for a multi-gene, next generation sequencing panel for hereditary cancer risk. Analysis was limited to 19 genes associated with hereditary breast and ovarian cancer (ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM, MLH1, MSH2, MSH6, NBN, PALB2, PMS2, PTEN, RAD51C, RAD51D, STK11, TP53). All patient demographic information was collected via a self-reported online health history questionnaire.

Results: In this cohort, the median reported age at breast cancer diagnosis was 49.3. Overall, 360 pathogenic or likely pathogenic variants were identified and reported. A total of 344 individuals were found to carry a single pathogenic mutation in one of the 19 genes analyzed, and 8 individuals were found to carry two concurrent pathogenic mutations in different genes. Notably, 173 of the 360 mutations identified were reported in the BRCA1 and BRCA2 genes (48.1%), yielding an overall BRCA1 and BRCA2 mutation carrier rate of 5.7%. Additionally, 12.2% of the cohort reported having bilateral breast cancer. The mutation carrier rate amongst these individuals was 15.0% (50/334), 60.8% of the mutations found in these individuals were in BRCA1 or BRCA2, and the average age at breast cancer diagnosis was 49.4 (43.3 amongst mutation carriers). In the entire cohort, a pathogenic mutation was identified in 15 of the 19 genes analyzed.

Conclusion:
In summary, the overall mutation carrier rate in this cohort for breast and ovarian cancer risk genes was 11.7% (352/3,011). Taken together, these data support the recommendation that all patients with breast cancer should undergo germline testing for hereditary cancer risk.
Background: Next-generation sequencing technology, reduced costs and public interest have fueled a surge in more expansive germline genetic testing of breast cancer patients. However, there are no population-based data on trends in use of different test types or the distribution of results.

Methods: In the iCanCare study, we surveyed 7,303 women diagnosed in 2013-15 with early-stage breast cancer and reported to the Georgia and Los Angeles County SEER registries. Of 5,080 respondents (response rate 70%), 5,050 were linked to SEER clinical data and to test results from four commercial laboratories that performed nearly all germline genetic testing for breast cancer patients in the regions. We examined trends in test type (two genes, BRCA1/2 only, vs. more cancer susceptibility genes, multigene panel) and patterns of results (positive for a pathogenic mutation; variant of uncertain significance (VUS); negative) by clinical and sociodemographic subgroups. Pre-test risk of having a pathogenic mutation was categorized as higher vs. average based on patient report of age at diagnosis, family cancer history, ancestry (Ashkenazi Jewish vs. not) and breast cancer subtype (triple-negative vs. not), according to practice guidelines criteria for genetic testing.

Results: The mean age was 62 years; 26%, 49% and 25% had stage 0, I, and II cancer, respectively; 78% had estrogen receptor-positive, HER2-negative disease, and 9% had triple-negative; 28% had higher pre-test risk of having a pathogenic mutation; 56% were non-Hispanic white, 18% African American, 14% Hispanic, and 10% Asian. Genetic testing use did not change over time (p=0.695), with one-quarter of patients receiving any test (either BRCA1/2 only or multigene panel) according to clinical laboratory data. However, testing included more genes over time: multigene panels comprised 19% of tests in 2013 vs. 66% in 2015 (p<0.001). Among all patients, 14% received BRCA1/2 only and 12% multigene panel testing, with no differences in test type by pre-test risk or race/ethnicity. Among all patients, 7% had a pathogenic mutation and 14% had a VUS in any gene. Patients at high pre-test risk had a lower ratio of uninformative VUS to informative pathogenic mutations (14%/10%) than average risk patients (15%/4%, p<0.001). There was a substantially higher ratio of VUS to pathogenic mutation among African Americans (22%/7%) and Asians (23%/3%) than other racial/ethnic groups (12%/8%, p<0.001).

Conclusions: In a large, diverse, contemporary sample of early-stage breast cancer patients accrued from population-based registries and linked to clinical laboratory data, one-quarter had genetic testing, with multigene panels markedly replacing BRCA1/2-only tests over time. The ratio of uninformative VUS to informative pathogenic mutation results was lowest in women at high pre-test risk and highest among racial/ethnic minorities. These findings can inform genetic counseling and they emphasize the urgent need to re-classify VUS results in racial/ethnic minorities. More research is needed to determine the impact of this marked increased in multigene panel testing on patient experiences, and the impact of test results on treatment decision-making and outcomes.
An interlaboratory study of complex mutation detection in genes associated with hereditary breast and ovarian cancer highlights both successes and current challenges

Stephen Lincoln¹, Justin Zook², Rebecca Truty¹, Shimul Chowdhury³, Andrew Fellowes¹, Shazia Mahamdallie⁵, Matthew Ferber⁶, Megan Cleveland², Catherine Huang⁷, Farol Tomson⁷, Eric Klee⁶, Wasanthi DeSilva⁶, Shelia Seal⁶, Swaroop Aradhya¹, Robert Nussbaum¹,², Russell Garlick⁷, Stephen Kingsmore³, Nazneen Rahman⁵, Mark Salit² and Brian Shirts⁹. ¹Invitae, San Francisco, CA; ²National Institute of Standards and Technology, Gaithersburg, MD; ³Rady Childrens Institute for Genomic Medicine, San Diego, CA; ⁴Peter MacCallum Cancer Centre, Melbourne, Australia; ⁵The Institute of Cancer Research, London, United Kingdom; ⁶The Mayo Clinic, Rochester, MN; ⁷SeraCare Life Sciences, Gaithersburg, MD; ⁸Volunteer Clinical Faculty, University of California, San Francisco, San Francisco, CA and ⁹University of Washington, Seattle, WA.

Background: Established laboratory methods, including next-generation sequencing (NGS), can accurately and inexpensively detect certain classes of clinically important mutations in readily accessible protein coding regions of a patient's DNA. These events include single nucleotide variants (SNVs), small insertions/deletions (indels), and sometimes medium sized copy-number deletions or duplications (del/dups). However, other mutation types with clinical relevance are known, and indeed data on over 80,000 patients show that pathogenic, medically important variants of other, more technically challenging types are prevalent in genes related to hereditary breast and ovarian cancer (see associated abstract by Lincoln et al.). We sought to develop and evaluate a reference standard by which laboratories may be able to assess and improve their performance on representative examples of complex, technically “hard” mutations in medically important genes associated with breast, ovarian, and other cancers.

Methods: We selected a diverse set of 23 challenging alterations, uncovered by the aforementioned clinical study, all of which are considered pathogenic and potentially actionable under ACMG and NCCN guidelines when uncovered in the germline DNA of a patient. These mutations are in the BRCA1, BRCA2, MLH1, MSH2, MSH6, and PMS2 genes. We generated a single synthetic DNA specimen with all 23 mutations introduced into a known genomic background. Following presentation of this general strategy at a 2016 meeting, 7 laboratories volunteered to collaboratively evaluate this approach. This DNA was created, validated, blinded and provided to these laboratories who sequenced it using a total of 9 different NGS based workflows, including 5 validated clinical tests with customized methods and two sequencing technology vendor (Illumina, Ion Torrent) default pipelines. Multiple target capture biochemical methods were used, as was whole genome sequencing.

Results: Twelve of the 23 variants were detected by all 9 laboratory workflows, but just 2 workflows detected all 23. Many, but not all, of these test limitations were previously known. Importantly, evidence of each variant was present in the raw data, suggesting that this strategy is compatible with the diverse biochemical methods used in NGS laboratories today. Raw data for the synthetic variants mimicked that of the endogenous ones (including presenting similar technical artifacts which make these variants “hard”) demonstrating that controls such as these may be useful in the development of methods with improved sensitivity. The vendor-supplied bioinformatics pipelines fared the worst, reinforcing the importance of carefully selecting bioinformatics algorithms and parameters in any laboratory developed test.

Discussion: Medically important but technically challenging mutations are prevalent in genes involved in hereditary breast, ovarian, and other cancers. Although patient specimens are also critical, synthetic controls may help efficiently assess the analytic range of any clinical test, highlighting certain strengths and limitations, and can help laboratories develop new methods to improve sensitivity for these challenging variants.
Addition of a remote genetic counselor to the breast specialist's team improves clinical decision-making

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Background: There is a shortage of trained genetic counselors (GC) and often long wait times for appointments, resulting in other specialists frequently ordering genetic testing. However, non-genetic specialists, including breast surgeons, find it difficult to stay current in genetics due to rapid advances in gene discovery, expanded panel offerings, and frequent changes to professional guidelines. We tested a novel model for hereditary cancer risk assessment where breast surgeons had “on demand” access to a remote laboratory-based genetic counselor for peer to peer consultation. In this study we sought to determine the impact this model has on breast surgeons' routinely ordering genetic testing including test identification, ordering patterns, and medical management.

Methods: An IRB approved multi-center prospective study involved 14 community-based breast cancer surgeons experienced with hereditary cancer risk assessment without a genetic counselor as part of their practice. Cases were all discussed with a remote Invitae GC to determine testing eligibility and selection. Physicians had the option to utilize remote GCs to discuss results or to refer to traditional genetic counseling services. Pre and post-test surveys were completed for each patient by the testing physician. To protect patient privacy, a unique case ID was used to link patient test data with identifying data.

Results: A total of 192 patients were evaluated with median age of 52. Risk assessment via BRCAPRO and the Hughes Risk model were performed on 98% of patients by the physicians. 65% of patients met NCCN guidelines for testing. Pathogenic mutations were found in 14% of patients. Breast surgeons changed their test selection 21% of the time after discussion with a GC. They called to discuss results in 47% of cases and medical management changes were incorporated in 15% of these cases based on discussion with a remote GC.

Conclusions: Remote GC provider support assisted physicians in facilitating customized test selection, aided in navigating challenging counseling cases, and impacted clinical management. This service may serve as a viable, effective model for 'on demand' genetic counseling support and may be a novel opportunity to expand genetic testing in a breast surgery setting.
Title: BRCA testing patterns at a safety net hospital and an academic medical center

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Body: Introduction
BRCA testing has become less expensive since elimination of patent claims and the advent of panel testing. However, criteria used to determine eligibility vary by insurance and testing remains costly for those with limited coverage. High risk patients may be under-tested or low risk patients over-tested. This study seeks to determine if BRCA testing was appropriately employed at safety net and academic sites.

Methods
Retrospective chart review was performed using a dataset obtained from the Georgia Tumor Registry of women ages of 20-70 diagnosed with breast cancer between 2010-2014 at Grady Memorial Hospital (GMH) and Emory University Hospital (EUH). Of 1142 EUH cases, 652 charts were randomly selected and all 532 GMH cases were reviewed. Demographic information collected included age, race, insurance status, and mean income based on zip code of residence. Family history, medical history, and tumor pathology were obtained from the electronic medical record. Records were reviewed for documentation of genetic counseling referrals, if testing was performed, and testing results. Low risk patients were classified as those who had no BRCA risk factors as determined by the National Comprehensive Cancer Network (NCCN) criteria for testing. Patients who met at least one NCCN criteria were classified as high risk. Data analysis was performed using univariate analysis and multivariable logistic regression.

Results
Data from 529 EUH charts and 468 GMH charts was included in the final analysis. Although 85% of patients referred for genetic counseling were high risk, only 38% of high risk patients underwent BRCA testing. High risk patients were tested more frequently than low risk patients (38% vs 8%, p <0.001), and patients ≤60 years old with triple negative breast cancer were tested more frequently than all others (39% vs 23%, p < 0.001). Patients ≤45 were tested more often than those > than 45 (48% vs 15%, p < 0.001). Patients ≤50 years old with additional risk factors such as concerning/unknown family history or a second primary breast cancer were not more likely to be referred for testing. Black women were more likely to be high risk than white women (60% vs 50%, p < 0.001) however white women were tested more often than black women (60% vs 50%, p < 0.001) however white women were tested more frequently (36% vs 18%, p < 0.001). EUH patients were more likely to be BRCA tested than GMH patients (OR 2.8, p < 0.001). BRCA testing rates were higher among women with private insurance and those with a higher median income. However, this difference was not statistically significant on multivariable logistic regression.

Conclusion
While analysis shows higher rates of genetic counseling referral and testing among high risk women, the percentage of high risk tested was suboptimal. Results suggest testing referrals are more readily triggered by easily identified risk factors of age and subtype compared to family/personal history. There are disparities in testing related to hospital site and race. Given provider overlap at sites studied, this points to possible gaps in access to testing and counseling. Providers may benefit from implementation of electronic prompts to improve guideline adherence. Further study is needed to test strategies to improve testing rates among high risk patients with inadequate insurance coverage or in resource-poor settings.
2017 San Antonio Breast Cancer Symposium

Publication Number: P4-06-11

Title: Is myriad a reliable genetic risk model for prediction BRCA1/2 mutations in Romanian high-risk breast cancer patients?

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Body: Aim: To assess the predictive efficacies of genetic risk model Myriad for BRCA1/2 mutations in high-risk Romanian breast cancer patients (pts).

Methods: This prospective study evaluated the validity of Myriad risk assessment model for 250 high-risk breast cancer pts tested for BRCA1/2 mutations between 02.2015-12.2016 at IOCN. Inclusion criteria selected pts diagnosed with triple negative breast cancer under the age of 50, or having conventional family history criteria. All pts signed an informed consent. Myriad calculator software was utilized in order to assess the score for all 250 patients. BRCA testing was performed using an AmpliSeq-based sequencing analysis, on the Ion Torrent Personal Genome Machine at RCFG. Pathogenic mutations were validated using Sanger technology. MLPA was performed for all pts.

Results: The mean Myriad scores for all patients was 8.09% (2.2-26.6%) and the mean Myriad score for BRCA1/2 mutated pts was 11.56% (2.2-26.6%). The majority of pts, 166 (66.4%) presented a Myriad score between 2.2-4.8%, out of which 18 pts (40.9%) were BRCA1/2 mutated. Considering a higher score and the presence of deleterious BRCA1/2 mutations, 12 (27.27%) pts presented a score of 10.3-10.4%, 9 (20.45%) pts a score of 21.2-21.9%, and respectively 5 (11.36%) pts had a score of 26.6%. The subgroup analyzes revealed that the prediction of Myriad genetic risk model in high-risk breast cancer pts was statistical significant when the groups present scores between 10.3 and 26.2%. Other subgroup analysis failed to demonstrate statistical significance.

Table 1: Frequency analysis according to Myriad genetic risk model scores and BRCA1/2 mutational status.

<table>
<thead>
<tr>
<th>Myriad genetic risk model scores* (n=250)</th>
<th>BRCA 1/2 mutated (n=44)</th>
<th>No BRCA1/2 mutation (n=191)</th>
<th>Variants of unknown significance # (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.2-4.8% (n=166)</td>
<td>18(40.9%)</td>
<td>136(71.20%)</td>
<td>12(66.66%)</td>
</tr>
<tr>
<td>6.9%-8% (n=8)</td>
<td>0</td>
<td>8(4.18%)</td>
<td>0</td>
</tr>
<tr>
<td>10.3-10.4% (n=45)</td>
<td>12(27.27%)</td>
<td>29(15.18%)</td>
<td>4(22.22%)</td>
</tr>
<tr>
<td>15.9%(n=1)</td>
<td>0</td>
<td>1(0.52%)</td>
<td>0</td>
</tr>
<tr>
<td>21.2%-21.9% (n=14)</td>
<td>9(20.45%)</td>
<td>4(2.09%)</td>
<td>1(5.55%)</td>
</tr>
<tr>
<td>26.6% (n=16)</td>
<td>5(11.36%)</td>
<td>10(5.23%)</td>
<td>1(5.55%)</td>
</tr>
</tbody>
</table>

* The risk was calculated using BRCA risk calculator available at http://www.myriadpro.com/brca-risk-calculator/calc.html # Number of VUS results includes 3 pts that presented BRCA mutations positive results.

Table 2: Myriad genetic risk model stratified by 10.3 and 21.2% scores and BRCA test results.

<table>
<thead>
<tr>
<th>Data analyzed</th>
<th>BRCA 1/2 mutated</th>
<th>No BRCA1/2 mutation</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myriad score 10.3%</td>
<td>12</td>
<td>29</td>
<td>41</td>
<td>0.0100</td>
</tr>
</tbody>
</table>
**Conclusions**: The Myriad genetic risk model can be an acceptable risk assessment tool for determining the risk of carrying BRCA mutations in Romanian population if the score is between 10-20%. The inaccuracy in carrier prediction using Myriad model represents a challenge worthy of additional investigation and comparison with other genetic models. Genetic counselors should recognize this limitation when using Myriad model and recommending genetic testing for Romanian high-risk breast cancer pts. ClinicalTrials.gov Identifier: NCT02317120.
Title: Different patterns of risk reducing decisions in affected or unaffected BRCA mutation carriers

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Body: BACKGROUND
Risk-reducing (RR) management decreases the risk of breast cancer and BRCA related gynecologic cancer. However, there are fewer reports on the RR management in Asia compared to Western countries. The aim of this study is to identify risk reducing management patterns with BRCA1 or BRCA2 mutation carriers.

METHODS
The study group consisted of all consecutive 1104 breast cancer, ovarian patients and their families of high-risk patients who underwent BRCA gene testing in National Cancer Center, Korea from 2008 to 2016. A total 220 BRCA mutations (19.9%) were detected with 125 (11.3%) of BRCA1 gene and 95 (8.6%) of BRCA2 gene.

RESULTS
Out of 220 BRCA mutations carriers, they were consisted of 83 breast cancers, 10 ovarian cancers, 7 both cancers, and 120 unaffected carriers. Among them, 42 were men and 178 were women. About 90% (198/220) had the familial history of breast, ovarian or both malignancies (113 in BRCA1 and 85 in BRCA2 mutation (p=0.821)). All 42 men chose surveillance. Among 178 female BRCA mutation carriers, 98 (55.1%) underwent risk reducing management including 18 (10.1%) of chemoprevention, and 80 (49.9%) had risk-reducing surgeries (RRSs) (1 case of risk-reducing mastectomy, 76 of risk reducing bilateral salpingo-oophorectomy (RRSO), and 3 of both) and 80 (54.9%) chose only intensive surveillance for both of breast and ovary cancer.

In affected carriers with breast cancer, 59 (71.1%) underwent RR management (1 case of risk reducing mastectomy, 53 of RRSO, 3 of both surgery, and 2 of chemoprevention). There was no risk reducing management in affected carrier with ovarian cancer patients. In 78 unaffected women carriers, 39 (50.0%) women received RR management (23 (29.5%) cases of RRSO and 16 (20.5%) cases of chemoprevention). The rates of RRSs have increased annually since the 2013 year, (prior to 2013 vs. since 2013, RRSs 28.6% (6 cases/21 carriers) vs. 37.2% (74/199), p<0.01).

CONCLUSION
This study was conducted on the largest numbers of BRCA mutation carriers in Asian countries. RRSO is the more preferred management for affected carriers with breast cancer or unaffected carriers. The results might be explained by the severity of the illness and that RRSO was only reimbursed RR strategy from the Korean Government Insurance. Tailored genetic counseling and insurance policy may enhance overall levels of RR management.
Title: BRCA1/2 mutations identified by screening a large unselected breast cancer cohort in Sweden

Jingmei Li1,2, Sean Wei Xiong Wen3, Mikael Eriksson1, Anders Kvist1, Helene N Christensen5, Astrid Torstensson5, Douglas F Easton6, Soo-Hwang Teo3, Åke Borg4, Henrik Grönberg1 and Kamila Czene1. 1Karolinska Institutet, Sweden; 2Genome Institute of Singapore, Singapore; 3Cancer Research Malaysia, Malaysia; 4Lund University, Sweden; 5AstraZeneca Nordic-Baltic, Södertälje, Sweden and 6University of Cambridge, United Kingdom.

Body: Background
Treatment options for BRCA1/2 breast cancer include new therapeutic agents, such as poly (ADP-ribose) polymerase (PARP) inhibitors, which selectively target BRCA defective cells. According to current Swedish screening guidelines, eligibility for clinical BRCA1/2 hereditary mutation testing is mainly based on family history of breast or ovarian cancer and early age onset. We aimed at examining the prevalence and characteristics of BRCA1/2 mutation carriers by screening a large unselected breast cancer cohort in Sweden, and comparing our results with BRCA mutation carriers already identified through the national BRCA testing program.

Methods
Germline DNA (blood) from 5122 women diagnosed with breast cancer between 2001-2008 (LIBRO1 study) were analysed for BRCA1/2 mutations by targeted sequencing (next generation sequencing, NGS), of which 5099 samples passed quality control. All patients provided informed consent. Information on patient and tumor characteristics was collected from the LIBRO1 database. Clinical BRCA testing information was obtained from the BRCA Lab (Lund University, Sweden), which carries out mutation screening for all oncogenetic clinics in Sweden. Multinomial logit models were used to compare tumor characteristics of BRCA1 and BRCA2 versus non-BRCA carriers. Multivariable logistic regression models were used to examine for differences between BRCA carriers identified through the national BRCA testing program and additional BRCA carriers found by sequencing the entire study population (not tested or not identified under current screening guidelines).

Results
In total, 92 (1.8%) BRCA1/2 mutation carriers were identified retrospectively by NGS. The prevalence of BRCA1/2 mutations was 1.6% (38/2363) between years 2001-2004; and 2.0% (54/2736) between years 2005-2008. After controlling for age and year of diagnosis, BRCA2 mutation carriers were in general similar to non-BRCA carriers regarding tumor characteristics (hormone receptor status, grade, tumor size and proliferation index), except for nodal involvement. BRCA1 mutation carriers, however, had more aggressive tumor characteristics than non-BRCA breast cancer patients. Overall, 55/92 BRCA1/2 mutation carriers (59.8%) found by NGS were not already identified through the national clinical BRCA testing program. The BRCA carriers identified by clinical testing were more likely high-risk individuals, i.e. younger, less likely to have experienced menopause, and more likely to be associated with a familiar ovarian cancer compared to those not identified through clinical testing, after adjusting for year of diagnosis. A larger proportion of BRCA2 (34/42, 80%) than BRCA1 mutations (25/50, 50%) were missed by selectively testing, mainly high-risk individuals.

Conclusion
BRCA1/2 mutations were found in approximately 2.0% of unselected BC patients. Six out of ten BRCA mutation carriers were not identified through the national testing program, which follows the screening guidelines. Revised guidelines might be needed for the effective identification of BRCA1/2 germline mutations.
Title: Voice of cancer patients: Association of family history with genetic mutations in breast and ovarian cancer

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There are specific guidelines for testing germline mutations in patients with breast cancer. Besides BRCA 1 and 2, germline mutations of multiple other genes are being recognized as risk factors for breast and ovarian cancers and hence more patients are being tested using multigene panels. Many patients share their experiences as freely shared messages in online forums. In this study, we use our automated system (VoCP) to analyze these messages to understand awareness of genetic testing in breast cancer patients and some factors that lead to their genetic testing.

Method: We collected 15.13 million unique messages (by 987,189 users) from 37 unrestricted cancer forums that provide clinically relevant information. We built custom ontologies for genetic tests; breast, ovarian and other cancers; risk reducing mastectomy and oophorectomy; and screening. We then used VoCP that uses techniques from Big Data Science and Artificial Intelligence (e.g., deep learning, topic modeling, information retrieval, and natural language processing) to extract relevant information from these messages.

Results: Out of 15.13 million, 30,074 messages from 7,838 distinct users were discussing genetic testing. Mutations that were discussed most include:

- BRCA1/BRCA2 (28,130 messages); 954 patients were positive
- PALB2 (218 messages); 114 were positive
- CHEK2 (315 messages); 149 were positive
- TP53 (180 messages); 55 were positive
- BRIP1 (76 messages); 36 were positive
- CDH1 (128 messages); 12 were positive
- NBN (80 messages); 28 were positive
- STK11 (48 messages); 2 were positive
- RAD50/ RAD51C (39 messages/48 messages)

Association with family history of cancer: 3,083 messages mentioned positive family history and some mentioned several cancers in their family; these messages discussed the following cancers:

- Breast cancer: 2,243 massages
- Ovarian cancer: 830 massages
- Colon cancer: 247 messages
- Pancreatic cancer: 121 massages
- Prostate cancer: 206 massages
- Lung cancer: 138 massages

Out of 954 BRCA 1 and 2 positive patients,
- 279 messages indicated their family members were tested for BRCA 1 and 2
- 156 messages indicated positive mutations were found in family members
- 23 messages indicated that male members of family were also being tested

Also, out of 954 BRCA 1 and 2 positive patients,
- 333 mentioned opting for bilateral mastectomy and 117 talked about getting Prophylactic Bilateral Salpingo Oophorectomy
- 309 messages talked about ovarian cancer once they found out that they have BRCA mutation

Discussion: There is increasing awareness of genetic testing and germline mutations in addition to BRCA 1 and 2 in breast cancer. Positive family history of cancer is mentioned in 10% of patients seeking genetic testing. VoCP reliably provides meaningful insights from patients' point of view to their treatment and their concerns; it also gives insight into unmet needs where more resources and research should be focused.

We plan to use VoCP for further segmenting these 30,074 messages to understand other attributes and awareness among such patients.
Title: Targeted sequencing of BRCA1/2 across a large unselected breast cancer cohort in Sweden

Jingmei Li1,2, Sean Wei Xiong Wen3, Mikael Eriksson1, Anders Kvist4, Helene N Christensen5, Astrid Torstensson5, Douglas F Easton6, Soo-Hwang Teo3, Åke Borg4, Henrik Grönberg1 and Kamila Czene1. 1Karolinska Institutet, Sweden; 2Genome Institute of Singapore, Singapore; 3Cancer Research Malaysia, Malaysia; 4Lund University, Sweden; 5AstraZeneca Nordic-Baltic, Södertälje, Sweden and 6University of Cambridge, United Kingdom.

Body: Background
Observed BRCA1/2 mutation frequencies can vary depending on the population screened, screening criteria, founder effects, and methods used for testing. We aimed at examining BRCA1/2 germline mutations, identified through targeted sequencing, in a large unselected breast cancer population in Sweden.

Methods
Sequencing libraries of germline DNA from 5122 breast cancer patients diagnosed between 2001-2008 (LIBRO1) were prepared (48.48 Fluidigm Access Array system, Fluidigm Corp, USA) and sequenced (Next-generation sequencing [NGS], Illumina HiSeq 2500, v2 chemistry, University of Cambridge, UK). A total of 5099 samples (97.8%) passed quality control and were analysed. BRCA1/2 carriers identified by NGS were compared with those who were already identified by clinical BRCA screening (n=418) performed by the BRCA Lab (Lund University). This unit carries out mutation screening for all oncogenic clinics in Sweden using denaturing high performance liquid chromatography (DHPLC) and multiple ligation-dependent probe amplification (MLPA).

Results
A total of 50 BRCA1 mutation carriers were identified, of which 28 were unique pathogenic germline mutations. Frameshift insertions and deletions made up 34/50 (68%) of the BRCA1 mutations. Exon 11 harbored 33/50 (66%) of the BRCA1 mutations. The most common mutation was c.3048_3052dupTGAGA (n=8), which is a founder mutation originating from the West coast of Sweden. Three other Swedish founder mutations were also identified (c.1082_1092del [n=5], c.3626delT [n=3] and c.2475delC [n=2]). For BRCA2, 42 mutation carriers were identified; 33 unique deleterious BRCA2 mutations (27 frameshift deletions, 3 frameshift insertions, 9 truncating and 3 splice sites). More than half of the mutations (24/42, 57%) were found on exon 11. Of the 418 women who had attended clinical BRCA testing, 38 deleterious mutations were found. Our screening method confirmed 34 of these mutations, as two each of BRCA1 and BRCA2 mutations were missed, since NGS was proven unsuitable for the detection of large exon duplications. NGS did, however, identify three more carriers not previously identified by clinical testing (c.3048_3052dup, c.2577delA and c.7442delT). Overall, 55/92 BRCA1/2 mutation carriers (59.8%) identified in the present study by NGS were not clinically tested.

Conclusion
NGS is comparable with current BRCA testing tools for the identification of BRCA1/2 germline mutations, suggesting that the technology has the potential to be used in BRCA1/2 clinical testing in unselected breast cancer patients.
2017 San Antonio Breast Cancer Symposium

Publication Number: P4-06-16

Title: Frequency of pathogenic mutation in patients at high risk for hereditary breast cancer

Hee-Chul Shin¹, Tae-Kyung Yoo³, Han-Byoel Lee², Hyeong-Gon Moon², Dong-Young Noh² and Wonshik Han². ¹Chung-Ang University Hospital, Seoul, Korea; ²Seoul National University Hospital, Seoul, Korea and ³Seoul St. Mary's Hospital, Seoul, Korea.

Body: Background: Next-generation sequencing technology allows the simultaneous sequencing of multiple target genes. We developed a gene panel containing 64 genes which were associated with various hereditary cancers. This study was performed to evaluate the frequency of pathogenic mutations associated with hereditary cancer among Korean patients at high risk hereditary breast cancer using multi-gene sequencing panel.

Methods: A total of 252 breast cancer patients with high-risk hereditary cancer were included. Among them, 179 patients (71.0%) had multiple primary cancers including breast cancer, 27 patients (10.7%) were diagnosed with bilateral breast cancer at age 40 or younger. Thirty-five patients (13.9%) had breast cancer family history of more than 2 relatives. With the 64-gene panel, sequence variants were detected by next-generation sequencing technology.

Results: Sixty seven patients (26.8%) were found to have 77 germline pathogenic mutations, 12 in BRCA1, 13 in BRCA2, 9 in CDH1, 3 in FH, 5 in MSH2, 2 in MSH6, 4 in NAT1, 6 in PTCH1, 3 in RAD51, 7 in RET, 4 in SPINK1, 3 in TP53 and one each in ALK, BRIP1, CHEK2, MLH2, MUTYH, and PTEN. In 20 patients (4.0%), 2 (n=9) or 3 (n=1) pathogenic mutations were detected. In 227 patients with BRCA1/2 negative, CDH1 (n=7), RET (n=7), PTCH1 (n=5), and MSH2 (n=5) were the most prevalent pathogenic mutations.

Conclusions: The 64 gene panel detected germline pathogenic mutations in 26.8% of Korean breast cancer patients with feature of hereditary cancer. Mutations of BRCA1, BRCA2, CDH1, RET, and PTCH1 were the most prevalent variants. Mutation carriers were considered as high risk to develop malignancy and recommended to receive genetic counseling and intensive cancer screening.
Body: Objective: We sought to investigate our center's experience with multi-gene panel testing in breast and ovarian cancer patients from a largely rural catchment area. Our goal was to identify predictors of pathogenic variants and assess indicators for expanded genetic testing.

Methods: We conducted a retrospective review of breast and ovarian cancer patients who underwent panel testing between May 2011 and April 2016. A variety of commercial gene panels were used with variant classification determined by the individual laboratory. Differences in patient demographics, age of first cancer diagnosis, number of genes tested and tumor characteristics including hormone/HER2 status, histology, differentiation, tumor size and AJCC staging classification were analyzed among pathogenic variant positive, negative, and VUS patient subsets with a chi-square test and one-way ANOVA.

Results: We identified 215 patients who underwent panel testing: the average age of patients was 52.9 ± 12 with first cancer onset at 47 ± 12; 27% of patients had undergone prior single-gene testing; 7% of patients had ovarian cancer and 93% of patients had breast cancer. VUS were detected in 18.1% of patients and pathogenic variants were detected in 9.3% of patients. Of the 20 pathogenic variants identified, 1 was detected in BARD1, 2 in BRIP1, 3 in MUTYH, 5 in CHECK2, 2 in BRCA1, 2 in BRCA2, 3 in ATM, 1 in PALB2 and 1 in PMS2. In our breast cancer cohort, there was a statistical difference (p=.03) between patients with VUS, pathogenic variants and negative testing in respect to hormone and HER2 status. Most pathogenic variants (75%) were in patients who were HER2 negative, with the majority of VUS detected in patients who were hormone receptor positive (66%). Between the groups, there were no differences in histology, tumor differentiation, size or AJCC stage classification. However, individuals with pathogenic variants tended to have a younger age of first cancer diagnosis, have higher grade disease and have triple negative tumors.

Conclusions: Specific tumor patterns (that is, HER2 negative or triple negative pathology) may be important indicators for genetic testing in breast cancer patients. Expanded panel testing should be considered in patients with a younger age of cancer diagnosis, higher grade disease and triple negative tumors.
Clinical application of multigene panel testing and genetic counseling for hereditary/familial breast cancer risk assessment: Prospective single center study

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Body: Background
The identification of individuals at elevated risk for hereditary cancers has allowed the development of consensus recommendations for cancer screening and prevention. The introduction of multigene panels may identify more individuals with breast cancer gene mutations than does testing for BRCA1/2 alone. Therefore, the multigenerational panel increase the need for genetic counseling suggesting preventive approach or cancer-specific screening to patients and family members. The rapid clinical introduction of multigene panel testing, however, have several issues such as low- to moderate-risk gene mutations and clinical recommendations. We collect the mutation results and clinical recommendations after testing with multigene panel and giving genetic counseling.

Methods
We had developed multigene panel consisted of 64 genes related to hereditary cancer through previous study and prospectively enrolled 104 individuals who were appropriate candidates for hereditary breast cancer evaluation. The patients were tested with 64-gene panel(Celemics) and results were provided by us 4~10 weeks later. We checked the family history of cancer and made a pedigree before testing.

Result
Among 104 participants, 26 patients harbored deleterious mutations, most commonly in high to moderate-risk breast/ovarian cancer genes (BRCA1/2, BRIP, RAD51 and RAD51D), Lynch syndrome gene(MSH6) and other genes(FH, SPINK1). We recommended the cancer-specific screening and/or preventive approach for mutation-positive patients and suggested additional genetic test for the family members. Among them, 6 (23%) patients received Risk reducing procedures (Prophylactic mastectomy or oophorectomy) and most of them(19 patients(73%)) received cancer specific screening.

Conclusion
We demonstrate the use of multigene panel testing for hereditary breast cancer and will suggest the process of the genetic counseling including indication and results analysis with multigene panel testing.
Title: Understanding factors associated with uptake of BRCA genetic testing among Orthodox Jewish women using a mixed-methods approach

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Body: Background: The prevalence of BRCA1/2 mutations among Ashkenazi Jews is 1 in 40. Compared to family history-based BRCA testing, population-based testing has been shown to detect more mutation carriers in this population. Orthodox Jews (OJ) are the largest and fastest-growing Jewish population in NY and represent a spectrum of observance including Modern Orthodox, Yeshivish, and Chassidic. This understudied population has unique social, cultural, and religious factors that may influence BRCA genetic testing. We examined factors influencing BRCA genetic testing decision-making and uptake among OJ women.

Methods: Using a mixed-methods approach, we conducted a cross-sectional online survey and 4 focus groups among OJ women in 5 communities in the NY/NJ area. The online survey included items on demographics, breast cancer risk factors, and validated measures of genetic testing intention/knowledge, breast cancer worry/risk perception, stigma, and religious/cultural factors affecting medical decision-making. Descriptive statistics and bivariate and multivariable logistic regression models were conducted. We conducted 4 focus groups with purposive sampling of women who responded to the survey. The qualitative analysis of the semi-structured focus group discussions further explored factors affecting BRCA genetic testing uptake.

Results: Among 321 evaluable survey participants, median age was 47 years (range, 25-82); 55.8% were Modern Orthodox, 30.5% Yeshivish, and 2.8% Chassidic; 84% were married; 6.2% and 0.6% had a history of breast and ovarian cancer, respectively. Although 57.6% had a masters or doctoral degree, only 37.7% had adequate genetic testing knowledge. Nearly 20% of the surveyed women had undergone BRCA genetic testing. After adjusting for known confounders, women who met family history criteria for BRCA genetic testing were nearly 10 times more likely to undergo genetic testing. Modern Orthodox compared to non-Modern Orthodox women and married compared to unmarried women were more likely to undergo genetic testing (odds ratio [OR]=2.31, 95% confidence interval [CI]=1.03-5.17; OR=3.49, 95% CI=1.03-11.80, respectively). Compared to Modern Orthodox women, non-Modern Orthodox women were more likely to consult with a rabbi or religious figure when considering genetic testing and other medical decisions. The focus group participants (N=31) confirmed the importance of rabbinic consultation in medical decision-making. Although stigma was not associated with genetic testing uptake in our survey data, it emerged as a prominent factor in decision-making among focus group participants due to its potential impact on marriageability and family.

Conclusions: We found that non-Modern Orthodox and unmarried women are less likely to seek BRCA genetic testing. Among non-Modern Orthodox women, rabbinic consultation was an important factor in genetic testing decision-making. By understanding the religious and cultural issues regarding genetic testing in the OJ community and by engaging faith-based leaders, we can develop culturally sensitive interventions designed to enhance knowledge and informed choice about BRCA genetic testing, which may facilitate the implementation of population-based genetic screening among Ashkenazi Jews.
Title: Assessment of the hereditary component in 94 cancer predisposition genes to triple negative breast cancer

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Body: Background: In women with triple negative breast cancer (TNBC) unselected for age or family history, 8-14% and 5% of patients harbor germline mutations in BRCA1 and BRCA2, respectively. Diagnosis of TNBC <60 years of age is one of the NCCN criterion for genetic testing of BRCA1 and BRCA2. The contribution of germline mutations in other cancer predisposition genes to TNBC, is, however, not well-studied.

Methods: TNBC was classified as tumors with <1% positively staining cells for ER and PR and HER2 = 0+, 1+ or 2+/not amplified. Genomic DNA was isolated from blood samples and targeted sequencing was performed using the TruSight Cancer panel (Illumina). Pathogenic mutations were identified using VariantStudio and classified as pathogenic, uncertain significance (VUS) or benign using ClinVar.

Results: 196 female patients diagnosed with TNBC 2001-2014 had genomic DNA available. Average age at diagnosis was 52.8 years (range 34.1-83.4 years). The majority of patients were of European (66%) or African (31%) American ancestry; 26% had a family history and 13% had died of disease with an average time to death of 2.81 years. Twenty-three (12%) of women with TNBC had pathogenic mutations in breast cancer genes BRCA1 (n=14), BRCA2 (n=5), PALB2 (n=1) and CHEK2 (n=3), two women had mutations in the colon cancer genes MUTYH, one had a mutation in the ovarian cancer gene BRIP1, and an additional three women had pathogenic mutations in cancer predisposition genes FANCD2, SDHB and XPC. An additional 42 women had VUS in 20 genes, including one in BRCA1 and 5 in BRCA2.

Discussion: Although the majority of pathogenic mutations in this cohort of women with TNBC were in the BRCA1 and BRCA2 genes (10%), panel testing allowed for the detection of mutations in other breast (2%), colon (1%), ovarian (1%) and other cancer (2%) predisposition genes. Panel testing thus identifies genes other than BRCA1/2 associated with increased risk of TNBC and may incidentally identify women who would benefit from enhanced surveillance for other cancers.

The opinions or assertions contained herein are the private ones of the author/speaker and are not to be construed as official or reflecting the views of the Department of Defense, the Uniformed Services University of the Health Sciences or any other agency of the U.S. Government.
The use of automated methods of breast density estimation to determine response to tamoxifen after one and after five years

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Body: Background
Previous studies have demonstrated that treatment with tamoxifen reduces breast density in a proportion of women. This is important since density reduction assessed visually is correlated with the long term preventative effect of tamoxifen (Cuzick J et al JNCI 2011). All reduction in risk was seen in women who had a 10% or more reduction in visually assessed percent density (PD) Here we investigate the value of automated techniques for estimating density reduction using estimates of change in dense volume (Volpara, V), and dense area (Densitas, D, Stratus, S) in comparison with the visual assessment of percent density.

Methods. Women at increased familial risk of breast cancer (n=135) aged 33-46 agreed to take tamoxifen for five years for breast cancer prevention. All were undergoing annual mammography as part of an early detection programme. Controls (n=204) were of the same age and risk, undergoing annual mammography in the same clinic. We report response data for each technique related to median change in dense volume or dense area after one year and 4-5 years of tamoxifen treatment.

Results. Median density in women taking tamoxifen at baseline and 1 year for V, D and S were 61.3 and 50cc, 43.5 and 35.0cm² and 48.7 and 31.8cm² respectively (p values all <0.001). The median and interquartile ranges for change in density from baseline to year 1 were 10.9cc (2.7-21.6), 6cm² (3.0-11.0) and 11.0cm² (3.7 to 19.8) respectively. The median change in percent density (PD) was 10% (5.0-15.0, p<0.001). Correlations of change between techniques for all women at 1 year ranged from 0.15 PD vs D (not significant) to 0.53 V vs S (p<0.001).

Thirty six women stopped tamoxifen at or before one year. Of these 14/36 (38.9%) had a significant increase in one or more subsequent density measures. Sixty-nine women have continued tamoxifen for 4-5 years to date: 29/69 (42.0%) had sustained reductions in density below the medians by all three automated techniques. In 30/69 (43.4%) there was a significant density reduction by one or two methods and in 10/69 (14.5%) there was no significant reduction familial by any method.

Discussion. This study confirms that tamoxifen produces marked reductions in density measured by automated techniques based on change in dense volume (Volpara), dense area (Densitas and Stratus) which can be used to assess response to tamoxifen in women on an annual mammography programme. However correlations between techniques were relatively low and suggests that more than one technique might be used in practice. Virtually all of the reduction in density seen at one year was sustained for as long as treatment continued. Whether the marked rise in density after early cessation of tamoxifen seen in some women is detrimental remains to be investigated. In summary, we demonstrate that change in density as a result of tamoxifen treatment may be evaluated by automated techniques which may be more applicable than visual techniques in the clinic.
Title: Understanding the mechanisms of action underlying the role of IL6ST, a key biomarker for prediction of response to endocrine therapy

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Body: Introduction: IL6ST is regarded as a putative ER target gene. Recently it has been recognised as a key biomarker for prediction of response to endocrine therapy (ET), having been included as the primary biomarker in our EA2Clin test and as an ER-signalling gene in the EndoPredict test. In both tests higher IL6ST expression is associated with a better response to ET and better prognosis. Despite its importance as a biomarker, little is known about its functional role in breast cancer (BC).

Methods: Pre- and on-treatment (at 14-days and at surgery) samples were collected from 102 post-menopausal women with ER+ BC, treated with 3-6 months of neoadjuvant ET. RNA was extracted for whole-genome expression analysis. From a subset with available fresh frozen tissue (28 patients, 83 samples) protein was extracted and proteome analysis using mass spectrometry is currently underway – results available for SABCS 2017. Immunohistochemistry was performed on FFPE tissue microarrays (TMAs) comprising pre-treatment samples from 102 patients. Cytoplasmic/membrane staining was scored using a graduated scale (0-3+) and nuclear staining was graded using an Immunoscore.

Results: IL6ST exists in membrane-bound and soluble forms of varying size. The full-length membrane bound molecule comprises 8 domains: 6 extracellular, 1 transmembrane and 1 cytoplasmic. In the EA2Clin test, pre-treatment BC tissues are stained for IL6ST with an antibody specific for a region spanning the transmembrane and cytoplasmic domains. TMAs were stained for IL6ST with both this and a second antibody binding the extracellular part, detecting both full-length and most soluble isoforms. Levels of both were correlated (R=0.82, P<0.0001).

IL6ST is known to mediate the action of cytokines including IL6, OSM and LIF via downstream regulation of pathways such as JAK/STAT. TMAs were stained for antibodies against IL6ST, OSM, IL6, total STAT3, pSTAT3 (Tyr705) and pSTAT3 (Ser727). IL6ST was scored as low (0/1+) or high (2+/3+). There was a positive association between levels of IL6ST and IL6 (P=0.02) and total STAT3 (P=0.003). There was no association between IL6ST and OSM or either pSTAT3.

Supervised gene expression analysis comparing pre-treatment samples with high and low IL6ST levels revealed increased levels of STAT3-regulated genes: cell cycle (CEBPD, CDKN1B), apoptosis (NFI3, ATF3, BCL2), extracellular matrix remodelling (ADM, SEPRINE1-3) and interferon signalling (IFIT1, IFI44, IFI27). Unsupervised gene enrichment analysis revealed increased expression of genes involved with JAK/STAT, PI3K, mTOR and ERBB1 signalling in tumours expressing higher IL6ST levels. Lower levels were associated with increased energy generation, cellular metabolism and epithelial-mesenchymal transition.

Conclusions:
• This is the first matched whole-genome and mass spectrometry proteome analysis of sequential ET-treated BC patients
• IL6ST predicts response to ET – it is used in 2 independent assays
• Levels of full-length IL6ST appear to be the most important for ET response prediction
• IL6ST may have an active role in BC cells, mediating signalling of cytokines such as IL6 through the JAK/STAT pathway and subsequent downstream transcriptional regulation.
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Title: EA2Clin: A novel immunohistochemical prognostic and predictive test for patients with estrogen receptor-positive breast cancer

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Body: **Background:** The majority of patients with early-stage estrogen receptor positive (ER+) breast cancer (BC) are treated with adjuvant endocrine therapy (ET) after primary surgery to reduce the risk of recurrence. A variety of tests are available to predict outcomes on ET but most require gene-level measurements and are expensive. Recently, we developed an immunohistochemistry (IHC) based test (EA2Clin) using levels of pre-treatment IL6ST together with clinical variables and on-treatment proliferation. The aim was to validate this test in cohorts of both pre- and post-menopausal women treated with two weeks of a variety of endocrine treatments (tamoxifen, fulvestrant or an aromatase inhibitor) prior to surgery.

**Methods:** The cohorts are: (A) 186 post-menopausal women (PMW) with ER+ BC treated with at least 2 weeks of preoperative or neoadjuvant letrozole or anastrozole, then surgery followed by adjuvant letrozole (n=132) or tamoxifen (n=54); (B) 51 pre-menopausal women (preMW) with ER+ BC treated with 2 weeks of either neoadjuvant tamoxifen (n=24) or one 750mg dose of faslodex (n=27), then surgery followed by adjuvant tamoxifen. The median follow-up was 5.4 years for cohort A and 10.2 years for cohort B. IHC analysis was performed using a Leica BOND III autostainer and the EA2Clin algorithm was used to stratify patients in binary high or low-risk groups.

**Results:** In the cohort of PMW, EA2Clin was highly significantly associated with both recurrence-free survival (RFS) (P<0.0001, HR=13.26, 95%CI=5.59-13.46) and breast cancer specific survival (BCSS) (P<0.0001, HR=12.93, 95%CI=4.43-37.72). The 5 and 10 year actuarial recurrence rates were 7%/22% and 46%/73% for the low and high risk groups, respectively. The actuarial breast cancer-related death rate for the low risk group was 5% at both 5 and 10 years, whereas for the high risk group was 33%/38%. Confounding factors were not found to be significant.

In the cohort of preMW, our test was significantly associated with both RFS (P=0.002, HR=5.71, 95%CI=1.91-17.05) and BCSS (P=0.016, HR=4.81, 95%CI=1.34-17.26). The 5 and 10 year actuarial recurrence rates were 12%/29% and 27%/77% for the low and high risk groups, respectively. The 5 and 10 year actuarial breast cancer-related death rates were 7%/19% and 9%/58% for low and high risk groups, respectively.

**Discussion:**
- This study has validated EA2Clin as the first predictive tool to incorporate clinical data with pre and on-treatment immunohistochemical biomarkers to predict accurately the outcome of patients with ER positive breast cancer treated with adjuvant ET.
- This test predicts both RFS and BCSS in pre- and PMW treated with a variety of endocrine agents.
- Because this test incorporates clinical variables with simple IHC, it can be performed locally in any pathology lab.
Title: The clinical utility of oncotype Dx for patients with recurrence scores of 10 or less: A value based pathology study of tumor histopathology and outcomes analysis in an integrated delivery and finance health system

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Body: Introduction
The majority of publications regarding breast cancer GEPTs rarely supply detailed breast tumor histopathology in their outcome studies. As a result, the cost effective role of clinical risk assessment with histopathology of breast carcinomas tends to be minimized. The aims of this study are to characterize the details of breast tumor histopathology of patients with Oncotype Dx Recurrence Scores (RS) of 10 or less, and determine if Oncotype Dx offers value and clinical utility for patients with these low grade tumors.

Methods
A total of 459 patients (18%) with Oncotype Dx RS of 10 or less were retrieved from a registry of 2558 patients with Oncotype Dx results. Patients had five years of follow-up with tumor registry and were treated with endocrine therapy alone. Tissue slides were available to review on 441/459 patients. Recorded details included (1) histopathologic type of carcinoma (2) mitotic score (MS), tubule formation, nuclear pleomorphism and Nottingham histologic (NG) grade. (3) Estrogen (ER) and progesterone (PgR) semi-quantitated by Allred Score and Histologic Score (H Score: strong 200-300, moderate 100-199, weak <100). (4) Lymph node status. (5) overall survival and breast cancer specific survival.

Results
Patient ages were 33-92, with mean/median age of 60, and all had endocrine therapy alone. 148 of 441 (34%) patients had carcinomas of “special types”, notable for low grade/good prognosis including tubular 22(15%), cribriform 15 (10.1%), papillary 17 (11.5%), and mucinous 28 (21%), along with 63 (42.5%) low grade classic lobular carcinomas and 3 (2%) low grade mixed ductal and lobular carcinomas. All 148 tumors had a MS of 1, were NG1 and had high ER HScores (280 median/263 mean) (Allred Scores 7-8) and high PR HScores (210 median/201 mean) (Allred Scores 6-8). The remaining 293 tumors were ductal carcinomas of no special type (NST), and 261/293 (89%) of these had a MS of 1/NG2. Of the remaining cases, 10 (3%) had a MS of 2/NG2, 18 (6%) had MS of 2/NG3 and four (1%) were MS3/NG3. Estrogen receptor H Score/Allred Score was strong (Allred Scores 7-8) in 395/441 (89.6%), moderate in 45 (10.2%) and weak in 1 patient (0.2%). Progesterone HScores were strong in (Allred Score 6-8) 269/441 (76%) and moderate in the remainder. Strong and moderate ER comprised 99.8% of tumors. Thus, tumors with MS1, and NG1, all with ER HScore ≥200 (Allred Score of 7-8) were enriched in the RS ≤10, and these features distinguished this group from other tumors with a MS1.

At 5 years, 433 patients (98%) were alive, 8 were dead, 1 from breast cancer due to distant recurrence. The 5-year breast cancer specific survival for this group was 99.7%. [95% CI 98.5-99.9,] 87 cases were accrued in the ongoing prospective study to date. There were 15/87 (17%) cases, 95% of which were correctly identified by pathologists as having an RS ≤10 using the criteria defined, with sensitivity 95%, specificity 86%, PPV 63% 95% CI(49.76-75.08), NPV 99% 95% CI(90.7-99.78). No patient had a recurrence score >22.

Conclusions
Pathologists can identify these low risk tumors with high accuracy. Oncotype Dx lacks clinical value and utility in this setting.
Impact of common polymorphisms of CYP19A1 and UGT2B17 gene deletion on early endocrine-responsive postmenopausal breast cancer

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Body: Background Polymorphisms of genes involved in estrogen production have been linked to breast cancer risk, prognosis and treatment response. Polymorphisms of the aromatase gene CYP19A1 influence its activity. The UGT2B17 catalyzes glucuronic acid transfer to a variety of substrates, including steroids and drugs like the aromatase inhibitor exemestane. We investigated the impact of two variants of CYP19A1 (rs10046, rs4646) and the UGT2B17 gene deletion on disease outcome in 125 postmenopausal women operated for ER-positive primary breast cancer enrolled in a randomized pre-surgical trial.

Patients Briefly, upon informed consent, postmenopausal patients with ER-positive breast cancer (stage T1–2, N0–1, M0) eligible for surgery were randomized to receive either exemestane (25 mg/day), or celecoxib (800 mg/day), or placebo for 6 weeks prior to surgery at the European Institute of Oncology (2004-2008). Exemestane showed a significant 10% absolute reduction in Ki67 labeling index compared to the other two arms. Serum and whole blood was taken at baseline and the day before surgery and stored at -80°C until assayed.

Methods DNA was extracted from blood by QIAamp DNA Blood Kits. The CYP19A1 rs1004/rs4646 were analyzed by Taqman genotyping assays in real-time PCR. The UGT2B17 deletion was estimated by copy number assay (Lifetechnologies). Serum estradiol (E2) and estrone (E1) levels were measured by gas chromatography tandem mass spectrometry detection (GS-MS/MS) after liquid-liquid extraction. The lower limit of quantitation were 0.625 pg/mL for estradiol and 1.56 pg/mL for estrone. The association of genetic polymorphisms with “any event” was assessed by the Cox proportional hazards models adjusted for confounders.

Results The genetic polymorphisms did not deviate from Hardy-Weinberg equilibrium (P-value ≥0.41) and minor allele frequency of rs10046 (A/G), rs4646 (C/A), and UGT2B17Del were 0.45, 0.22, and 0.31, respectively. The rs10046 A and rs4646 C alleles were associated with higher estrogen levels. Carriers of rs10046 AA had median levels of 7.57 pg/ml E2 and 35.9 pg/mL E1 versus 3.9 pg/mL E2 and 27.4 E1 pg/mL in CA/AA genotypes (P<0.003). Carriers of rs4646 CC had 5.6 pg/ml E2 and 30.45 pg/mL E1 versus 3.95 pg/ml E2 and 27.4 E1 pg/mL in CA/AA genotypes (P=0.05 only for E1). After 6 weeks treatment with exemestane, we observed steeper decreases in estrogen levels in the rs10046 AA/rs4646 CC carriers (P=0.02 for E2). After a median follow-up of 7 years we found that women carrying at least one SNP of rs10046 and one SNP of rs4646 had a better prognosis compared with women carrying homozygote wt SNPs (HR=0.44; 95% CI: 0.2-0.99 P=0.049). Similarly, the UGT2B17 deletion was associated with a better prognosis (HR= 0.43; 95% CI: 0.19-0.97; P=0.0439). There was no interaction with pre-surgical or adjuvant treatment.

Conclusions Our analysis confirms previous findings of an association of CYP19A1 rs10046/rs4646 with estrogen levels in postmenopausal women. Interestingly, the carriers of the variants associated with lower estrogen levels at diagnosis had better prognosis. Further genomic profiling in larger trials aimed to enhance tailored treatment efficacy in endocrine-responsive postmenopausal breast cancer are warranted.
Title: Phosphorylation of insulin-like growth factor-1 receptor (IGF-1R) is associated with tamoxifen resistance by activating the PI3K/MAPK pathway

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Body: Background
IGF-1R is overexpressed in a substantial number of breast cancer cases. When phosphorylated, IGF-1R activates the PI3K and MAPK pathways. In preclinical studies, IGF-1R overexpression has been described to be associated with tamoxifen resistance. Expression of activated IGF-1R has not been established as a marker for endocrine resistance in the clinic. We tested the value of p-IGF-1R to predict adjuvant tamoxifen benefit in IGF-1R-positive tumors from ER+ breast cancer patients and its possible association with PI3K/MAPK activation. We also carried out cell line experiments to illustrate a potential causal link between IGF-1R activation status, PI3K/MAPK pathway activation and tamoxifen resistance.

Methods
Primary tumor blocks from 563 ER+ (stage I-III) postmenopausal patients previously randomized between tamoxifen (1 to 3 years) vs. no adjuvant therapy (IKA trial 1982-1993; Beelen et al, Breast Cancer Res, 2014) were recollected. Immunohistochemistry scoring on tissue microarrays included PTEN, p-IGF-1R, p-AKT(Thr308), p-AKT(Ser473) and p-p70S6K(Thr389) by cytoplasmic intensity (0-3), of p-4EBP1(Ser65) and p-ERK1/2(Thr202/204) by percentage of tumor cells with positive nuclear staining and of p-S6RP(Ser235/236) by percentage of tumor cells with positive membranous staining. Informative data on p-IGF-1R staining was available for 364 IGF-1R-positive tumors. Multivariate Cox models including standard prognostic factors were used to assess hazard ratios (HR) for recurrence-free interval for tamoxifen treatment, p-IGF-1R status (negative vs. positive) and their interaction. MCF-7 and T47D cell lines were used to validate the clinical findings. IncuCyte cell proliferation experiments were performed with various IGF-1R-related growth stimulating and inhibiting conditions. Western blots were carried out on cells under various growth conditions to analyze whether activation of IGF-1R would be associated with PI3K/MAPK pathway activation.

Results
Patients having tumors without p-IGF-1R expression derived significant benefit from tamoxifen (HR 0.4290, 95% CI 0.2356 - 0.7813; p = 0.00566), while those having tumors with p-IGF-1R expression had no benefit (HR 0.8051, 95% CI 0.2643 – 2.453; p = 0.70). The p for interaction was not significant (p = 0.32106). Tumors positive for p-IGF-1R had more expression of (phospho)proteins downstream of the PI3K/MAPK pathways. These results were supported by Western blots from the cell lines examined under various growth conditions. In both cell lines, linsitinib (a dual IGF-1R and insulin receptor inhibitor) was able to block IGF-1R signaling, preventing activation of the PI3K and MAPK pathways and abrogating cell proliferation in the presence of tamoxifen.

Conclusions
Postmenopausal breast cancer patients with p-IGF-1R-positive tumors appear to derive no benefit from adjuvant tamoxifen. Tumors with p-IGF-1R expression were associated with PI3K/MAPK pathway activation. In breast cancer cell lines with activated IGF-1R signaling, addition of linsitinib to endocrine therapy can restore sensitivity. This combination might be an interesting treatment option for tamoxifen-resistant patients.
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**Title:** PIK3CA mutation, reduced AKT serine 473 phosphorylation, and increased ERα serine 167 phosphorylation are positive prognostic indicators in postmenopausal estrogen receptor-positive, HER2-negative early breast cancer

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**Body:** Background: Endocrine therapy is the most important treatment option for women with estrogen receptor (ER)-positive breast cancer. We recently reported that approximately two-thirds of patients who relapsed within 5 years had received anthracyclins and/or taxanes as adjuvant or neoadjuvant chemotherapy in addition to adjuvant endocrine therapy. New strategies, such as signal transduction inhibitors together with endocrine therapy are required to improve survival. PIK3CA mutations are detected in almost 40% of early ER-positive breast cancers, and are therefore the most frequent genetic alterations in this subtype. PIK3CA mutation status is reported to affect activation of AKT and ERα. Moreover, recent studies demonstrate that patients had a better prognosis when tumors expressed ER, androgen receptor (AR), and vitamin D receptor (VDR).

Methods: Expression of AR and VDR, phosphorylation of AKT serine (Ser) 473 (AKT phospho-Ser473) and ERα Ser167 (ERα phospho-Ser167) were examined by immunohistochemistry in ER-positive, HER2-negative early breast cancer tissues. Seventeen mutations in exons 1, 4, 7, 9, and 20 of the PIK3CA gene were detected in genomic DNA extracted from formalin-fixed paraffin-embedded tumor blocks. Correlations between these biological markers and clinicopathological factors and prognosis were analyzed separately in pre- and postmenopausal women.

Results: Levels of AKT phospho-Ser473 were significantly higher in premenopausal women (n = 62) than in postmenopausal women (n = 152) (P < 0.0001 and P = 0.014, respectively). In contrast, expression levels of AR were significantly higher in postmenopausal women than in premenopausal women (P < 0.0001). In premenopausal women, 26 tumors (43%) had a single mutation of PIK3CA gene, and 3 tumors (5%) had mutations at two sites. In postmenopausal women, 64 tumors (44%) had a single PIK3CA mutation, 6 tumors (4%) had mutations at two sites, and one tumor (1%) had mutations at three sites. In premenopausal women, wild type PIK3CA was associated with smaller tumor size, higher ER expression levels, and lower AR expression levels when compared with women in the same cohort with PIK3CA mutant tumors. In postmenopausal women, patients with PIK3CA wild-type tumors had higher Ki67 labeling index, higher AKT phospho-Ser473, and lower ERα phospho-Ser167 when compared to patients with PIK3CA mutant tumors. Postmenopausal women with PIK3CA wild-type tumors had significantly worse disease-free survival than patients with PIK3CA mutant tumors (P = 0.007). In contrast, PIK3CA mutation status was not correlated with survival in premenopausal women. Low levels of AKT phospho-Ser473 and high levels of ERα phospho-Ser167 were strongly associated with increased disease-free survival in postmenopausal women (P = 0.016 and P = 0.0016, respectively).

Conclusion: ERα activation, in addition to PIK3CA mutation, may be biomarkers for highly endocrine-responsive tumors. This would facilitate the selection of postmenopausal ER-positive breast cancer patients who are likely to benefit from endocrine therapy alone from those who are not.
Title: Biomarkers to predict distant recurrence free survival after neoadjuvant endocrine therapy in breast cancer. A long follow up retrospective study

Miguel Gil-Gil¹, Idoia Morilla¹, Anna Petit¹, Teresa Soler¹, Xavier Perez-Martin¹, Anna Guma¹, Maria Jesus Pla¹, Raul Ortega¹, Amparo Garcia-Tejedor¹, Catalina Falo¹, Robert Montal¹², Luis Perez-Casanova¹, Carolina Loayza¹ and Sonia Pernas¹. ¹Institut Català d’Oncologia, Medical Oncology (ICO) - Hospital Universitari de Bellvitge (HUB). Breast Cancer Unit, L’Hospitalet, Barcelona, Spain and ²Hospital Clinic, Barcelona, Spain.

Body: Background:
Neoadjuvant endocrine therapy (NET) is gaining more acceptances for the management of estrogen receptor (ER) positive breast cancer (BC). Rate of patients achieving pathological complete response is very low and Ki67 suppression and PEPI score are the only prognostic factors associated with relapse free survival.

The aim of our study was to identify biomarkers of prediction of distant relapse risk that could help clinicians in the decision-making of systemic adjuvant treatment in patients previously treated with NET

Material & Methods:
Retrospective study of 119 postmenopausal women with ER or progesterone receptor (PR) positive BC treated with NET in ICO-HUB from 1997 to 2009. Clinical-pathological data and treatments administered were reviewed. IHC expression of ER, PR, Ki67, Androgen receptor (AR), BCL-2, Cyclin D1 (CD1), p16, p53, CD 44 and synaptophysin were analyzed in post-NET surgical formalin-fixed paraffin-embedded tumor samples through a tissue microarray. Survival was calculated by Kaplan-Meier method. Univariate and multivariate analysis of variables associated with distant relapse free survival (DRFS) was evaluated by Cox proportional hazard model.

Results:
Mean age was 74 (63-88). cT: T2 5%, T3 6.5%, T4 43.5%. cN: N0 59%, N1 25%, N2-3 16%. Stage: I 21%, II 49.5%, III 29.5%. Histological subtype: ductal 84%, lobular 6%, others 10%. Histological grade: G1 20%, G2 55%, G3 25%. Vascular invasion 15%. NET: Aromatase Inhibitors 64%, SERM 36%. Median duration of NET 8.5 months. Clinical Response: Complete 4%, Partial 55%, Stable 37%, Progression 4%. Surgery: Lumpectomy 72%, Mastectomy 28%;Lymphadenectomy 70.5%, Sentinel lymph node biopsy 6%, No surgical approach of axilla 23.5%. Surgical specimen: ypT1 36%, ypT2 54%, ypT3 6%, ypT4 4%; ypN0 28%, ypN1 22%, ypN2 13.5%, ypN3 12% ypNx 23.5%. Surgical margins: Negative 89% Positive 11%. Median fibrosis rate 20% (0-95). PR and Ki67 showed a statistically significant decrease after NET(p<0.05) but no ER (p=0,29). Adjuvant treatment: chemotherapy 7%, radiotherapy 76%, endocrine therapy 96%. Median follow-up: 104 months. Only 21 patients developed distance relapse. Median OS was 139 months [95% CI = 98-181]. Univariate analysis for DRFS showed statistically significant differences in cN (HR=3.61), histological grade 3 (HR=3.62), ypN (HR=3.62), p16 (HR=6.1) and p53 (HR=2.79). Multivariate analysis of post-NET biomarkers showed that negative nuclear p16 expression (HR=4.79)and positive p53 (HR=2.83)were independently associated with worse DRFS. In multivariate analysis of all clinico-pathological and molecular factors, histological grade 3 (HR=2.82) was the sole DRFS independent factor.

Conclusions:
Negative nuclear p16 expression and positive p53 post-NET were associated with worseDRFS. When all clinico-pathological and molecular factors were analysed, G3 was the sole DRFS independent factor. Patients with G3, negative p16 or positive p53 after NET could probably benefit from adjuvant chemotherapy or CDK 4-6 inhibitors treatment. In our series, we did not find usefulness in analysing ER, PR and Ki67 post-NET changes to predict DRFS.
Title: A multi-study correlative analysis of change in Ki67 in the neoadjuvant setting and disease free survival improvement in the adjuvant setting

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Body: Background: Based on results from the ATAC and IMPACT trials, Dowsett et al.¹ hypothesized that “short-term changes in proliferation in the neoadjuvant setting may be able to predict outcome during adjuvant use of the same treatments.” Recent neoadjuvant studies²,³,⁴ of novel agents for HR+ breast cancer used Ki67, a biomarker for cell proliferation, as an endpoint for evaluating biological activity. However, the potential of this endpoint for predicting the adjuvant efficacy of therapeutic agents remains highly uncertain, motivating continued study based on clinical data. After curating relevant data from multiple trials (in both the neoadjuvant and adjuvant settings), a statistical model was constructed to evaluate the current evidence regarding this hypothesis.

Methods: Data were collated from randomized trials of systemic therapies in post-menopausal women with HR+ breast cancer reporting Ki67 reduction in the neoadjuvant setting (7 studies) or disease-free survival (DFS) in the adjuvant setting (5 studies). A Bayesian hierarchical joint network meta-analysis model was constructed and fit to the data to evaluate: (1) if there is an association between study-level neoadjuvant Ki67 reduction for a given regimen and corresponding adjuvant DFS hazard ratio, and (2) if the relationship does exist, what DFS effect does it predict for various regimens for which only neoadjuvant data are currently available.

Results: The results of the statistical model indicate that effect-size based on neoadjuvant Ki67 correlates with adjuvant (DFS) hazard ratio. Using neoadjuvant Ki67 data for several therapies for which adjuvant data are presently unavailable, we demonstrated how forecasts (and associated confidence intervals) of adjuvant effect-size may be produced using the model.

Conclusions: While randomized trials yielding paired data from both clinical settings are sparse, this assessment using a statistically rigorous approach sheds additional light on the hypothesis of a correlation between neoadjuvant Ki67 changes and adjuvant DFS improvements. This analysis suggests the potential utility of Ki67 as a surrogate endpoint to screen/prioritize experimental regimens for development in the adjuvant setting.

References:
**Title:** Disseminated tumor cells as predictive factor of benefit of lymph node irradiation to prevent loco-regional relapse

Francois-Clement Bidard,1,2 Fabien Mignot,1 Philip Poortmans,1 Sylvain Dureau,1 Frederique Berger,1 Delphine Loirat,1 Charlotte Proudhon,1 Anne Vincent-Salomon,1 Jean-Yves Pierga1 and Youlia Kirova1,3. 1Institut Curie, Paris, France; 2Versailles Saint Quentin University, Paris Saclay University, Saint Cloud, France and 3Paris Descartes University, Sorbonne Paris Cite University, Paris, France.

**Background:**
Early stage breast cancer patients with micrometastatic spread (cM0(i+) per the 2010 TNM staging), detected either in the bone marrow (disseminated tumor cells, DTC) or in the blood (circulating tumor cells, CTC) are at higher risk of distant relapse and death. Loco-regional relapses were also more frequently observed in patients with DTC and, recently, with CTC (IMENEO study, Bidard et al, SABCS 2016). In that context, we analyzed whether DTC detection would be a predictive factor for the benefit of comprehensive loco-regional irradiation.

**Methods:**
Patients with localized breast cancer were eligible for this IRB-approved prospective cohort after informed written consent. DTC status was prospectively assessed by trained pathologists after immunocytostaining following ISHAGE criteria, at time of surgery or prior to any primary systemic therapy. Irradiation volumes (breast or chest wall +/- regional lymph nodes) were defined per standard of care. Loco-regional relapse was defined as documented ipsilateral invasive relapse occurring in the breast, chest wall and/or in regional lymph nodes, prior to any distant metastatic relapse. Loco-regional relapse-free interval (LRFI) was defined as the time elapsed between breast surgery and locoregional relapse. Cumulative incidence rates and hazard ratio were obtained using both Cox and Fine-Gray models, taking into account metastatic relapse and death as competitive events. Interaction tests were performed to confirm the predictive value of DTC status in a multivariate analysis.

**Results:**
From 11.1998 to 09.2005, a total of 620 patients with non-metastatic breast cancer were included in this prospective cohort. Median FU was 11.7 years. Overall, 94 patients (15.1%) were DTC-positive and 50 patients (8.1%) experienced a locoregional relapse during follow-up. DTC detection was significantly associated with shorter LRFI in univariate and multivariate analyses (Cox, HR=2.6 [1.4;4.8], p=0.004 ; Fine-Gray, HR=1.76 [1.04;3.0], p=0.04). In the multivariate subgroup analysis, locoregional lymph node irradiation was associated with a longer LRFI for DTC-positive patients, but not for DTC-negative patients (interaction test in multivariate analysis: p=0.03). Similar results were obtained when taking locoregional relapses synchronous with distant metastatic disease into account (interaction test: p=0.02). Importantly, the predictive value of DTC status for the benefit of locoregional irradiation was independent of other clinical and pathological characteristics, including locoregional nodal (pN) status.

**Conclusion:**
This long term analysis confirms the independent long-term prognostic value of DTC on locoregional relapses. Moreover, the finding that cM0(i+) status is a predictive marker for the efficacy of locoregional lymph node irradiation promises a new opportunity to better tailor adjuvant radiation therapy in early stage breast cancer patients.
Title: On the development and clinical value of RNA-sequencing-based classifiers for prediction of the five conventional breast cancer biomarkers: A report from the population-based multicenter SCAN-B study

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Body: Background:
In early breast cancer, five histopathological biomarkers are part of current clinical routines and used for determining prognosis and treatment: estrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor receptor 2 (ERBB2/HER2), Ki67, and Nottingham histological grade (NHG). We aimed to develop classifiers for these biomarkers based on tumor mRNA-sequencing (RNA-seq), compare classification performance to conventional histopathology, and test whether RNA-seq-based predictors could add value for patient risk-stratification.

Patients and Methods:
In total, 3678 breast tumors were studied. For 405 breast tumors in the training cohort, a comprehensive histopathological biomarker evaluation was performed by three pathology readings to estimate inter-pathologist variability on the original diagnostic slides as well as on repeat immunostains for this study, and the consensus biomarker status for all five conventional biomarkers was determined. Whole transcriptome gene expression profiling was performed by RNA-sequencing on the Illumina platform. Using RNA-seq-derived tumor gene expression data as input, single-gene classifiers (SGC) and multi-gene classifiers (MGC) were trained on the consensus pathology biomarker labels. The trained classifiers were tested on an independent prospective population-based series of 3273 primary breast cancer cases from the multicenter SCAN-B study with median 41 months follow-up (ClinicalTrials.gov identifier NCT02306096), and classifications were evaluated by agreement statistics and by Kaplan-Meier and Cox regression survival analyses.

Results:
For the histopathological evaluation, pathologist evaluation concordance was high for ER, PgR, and HER2 (average kappa values of .920, .891, and .899, respectively), but moderate for Ki67 and NHG (.734 and .581). Classification concordance between RNA-seq classifiers and histopathology for the independent 3273-cohort was similar to that within histopathology assessments, with SGCs slightly outperforming MGCs. Importantly, patients with discordant results, classified as hormone responsive (HoR+) by histopathology but non-hormone responsive by MGC, presented with significantly inferior overall survival compared to patients with concordant results. These results extended to patients with no adjuvant systemic therapy (hazard ratio, HR, 4.54; 95% confidence interval, CI, 1.42-14.5), endocrine therapy alone (HR 3.46; 95% CI, 2.01-5.95), or receiving chemotherapy (HR 2.57; 95% CI 1.13-5.86). For HoR+ cases receiving endocrine therapy alone, the MGC HoR classifier remained significant after multivariable adjustment (HR 3.14; 95% CI, 1.75-5.65).

Conclusions:
RNA-seq-based classifiers for the five key early breast cancer biomarkers were generally equivalent to conventional histopathology with regards to classification error rate. However, when benchmarked using overall survival, our RNA-seq classifiers provided added clinical value in particular for cases that are determined by histopathology to be hormone-responsive but by RNA-seq appear hormone-insensitive and have a significantly poorer outcome when treated with endocrine therapy alone.
Title: High levels of Bruton's tyrosine kinase (BTK) protein is associated with favorable outcome in breast cancer

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Body: Background: Bruton's tyrosine kinase (BTK) belongs to the TEC family of cytoplasmic tyrosine kinases. BTK promotes survival and proliferation of malignant B-cells. Although BTK is predominantly cytoplasmic in hematopoietic malignancies, BTK may also be localized to cell nuclei. In breast cancer cell lines, BTK was identified as a candidate survival factor based on a large-scale loss-of-function analysis of human tyrosine kinases using RNA interference. We therefore sought to determine whether BTK levels in breast cancer cells were associated with clinical outcome.

Methods: For these studies we applied quantitative immunofluorescence-based immunohistochemistry of breast cancer tissue specimens in tissue microarray (TMA) format. All patients underwent upfront breast surgery; hence there are no confounding effects of neoadjuvant treatment on biomarker levels. Within the cancer cell compartment, BTK protein was expressed primarily in nuclei of scattered breast cancer cells. Because of the scattered BTK expression distribution, we developed an analytical approach that considered all percentiles of cell-by-cell BTK protein expression in nuclei of all cancer cells sampled within a tumor tissue. Tumors from patient Cohort A (training set, N=758) was used to define the optimal dichotomized (high vs low) BTK marker by selecting the percentile that dichotomized to have the highest predictive power for progression free survival (PFS). The prognostic value of the BTK marker was subsequently evaluated in tumors of the independent validation Cohort B (N=316) using Kaplan-Meier curves and the Cox proportional hazards model for PFS.

Results: The analyses of the training set identified the 94th percentile of BTK cell-by-cell distribution as the optimal predictor of recurrence when dichotomized as High if Qₙ(0.94)>0.369 and as Low if Qₙ(0.94)≤0.369. For tissue cores in the training set, 467 of 758 (61.6%) were classified as High BTK and 291 of 758 (38.4%) were classified as Low BTK. Application of the optimal cut point from Cohort A to tumors in the validation Cohort B classified 145/316 cores (45.9%) as High BTK and 171/316 cores (54.1%) as Low BTK. High BTK was associated with longer PFS in both training (p=0.001) and validation sets (p=0.048). The result remained similar and significant in the final multivariate Cox model (HR=0.50; 95% CI 0.26-0.97; p=0.039) after adjusting for pathology and treatment variables.

Conclusions: High BTK nuclear protein expression was associated with a significantly more favorable clinical outcome in breast cancer patients, and held up as an independent marker after adjusting for other variables, including hormone receptor status and treatment. Although the association of High BTK is associated with favorable outcome, further studies are warranted to determine whether BTK represents a therapeutic target in breast cancer.
Title: Validation of 12-gene chemokine signature as a predictor of treatment response in breast cancer

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Body: Background: We had previously derived a unique 12-chemokine gene expression score (CS) from a metagene grouping with high enrichment for immune-and inflammation-related genes. A review of selected Stage I - III breast cancer patients showed that higher CS were associated with high-grade tumors and aggressive subtypes and in the HER2 positive group, correlated with increased recurrence free survival that trended towards significance. We tested the predictive capability of this CS for pathological complete response (pCR) in an external dataset. We used the Neratinib and Veliparib arms of the I-SPY2 TRIAL dataset with their respective controls for this analysis.

Methods: Gene expression signature probes (CCL2, CCL3, CCL4, CCL5, CCL8, CCL18, CCL19, CCL21, CXCL9, CXCL10, CXCL11 and CXCL13) were extracted from existing Agilent custom 44k microarray from the I-SPY2 TRIAL dataset. The arrays contain 40,793 probe sets representing ~25,000 unique genes. The expression data for the 246 distinct solid tumors were normalized using IRON and expression data for the 12-chemokine genes were extracted for principal component analysis (PCA). The first principal component (PC1, explaining ~57%) was calculated using R package. The median CS of 0.79 was used as the cut-off with any score above this defined as high and scores at or below the median were classified as low. The Chi-Square test or Fisher's exact test was used to test pCR vs CS within each treatment arm [table 1]. Cochran-Mantel-Haenszel test was performed to test the pCR for the pooled control and treatment arms between CS high and low groups adjusting for hormone receptors (HR), HER2 and Mammaprint status. Breslow-Day test was performed to test treatment difference in the odds ratios for the CS and response.

Results: There were 56 patients in the paclitaxel arm (A), 115 in the Paclitaxel+Neratinib arm (B), 22 patients on the Paclitaxel + Trastuzumab arm (C) and 72 on the Paclitaxel + Veliparib + Carboplatin arm (D). In all treatment arms, high CS were associated with higher pCR rates with significant association found in treatment arms A and D (38.5% vs 6.7% and 47.5 vs. 25% respectively)[table 1]. Analysis of pooled data for all arms adjusting for HR, HER2 and Mammaprint status showed statistically significant association between CS and pCR (P < 0.05). There were no significant differences in the odds ratios for the CS and pCR.

Conclusion: The 12 gene CS predicted for treatment response even after adjusting for the treatment with no differences noted in the odds ratio for CS and pCR. The 12 gene CS can be readily obtained from I-SPY2 TRIAL microarrays to characterize tumors immunologically and possibly predict response to novel therapies. Continued investigation of the CS in other I-SPY2 TRIAL treatment arms is warranted.

Table: 1 Comparison of treatment arms with gene scores and treatment response

<table>
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<th>12 gene score</th>
<th>pCR N(%)</th>
<th>Incomplete Response N(%)</th>
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<td>Low</td>
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<td></td>
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<td>7 (63.6)</td>
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Title: A targeted breast cancer radiosensitivity gene expression panel

Martin Sjöström¹,², Johan Staaf¹, Patrik Edén⁴, Fredrik Wärnberg⁴,⁵, Jonas Bergh⁶,⁷, Per Malmström¹,², Mårten Fernö¹, Emma Niméus¹,⁸ and Irma Fredriksson⁹,¹⁰. ¹Lund University, Clinical Sciences Lund, Oncology and Pathology, Lund, Sweden; ²Skåne University Hospital, Lund, Sweden; ³Lund University, Computational Biology and Biological Physics, Lund, Sweden; ⁴Uppsala University, Uppsala, Sweden; ⁵Akademiska University Hospital, Uppsala, Sweden; ⁶Karolinska Institutet, Cancer Center Karolinska, Stockholm, Sweden; ⁷Karolinska University Hospital, Radiumhemmet, Stockholm, Sweden; ⁸Skåne University Hospital, Lund, Sweden; ⁹Karolinska Institutet, Stockholm, Sweden and ¹⁰Karolinska University Hospital, Stockholm, Sweden.

Body: Background: A majority of patients with early breast cancer is operated with breast conserving surgery (BCS) and adjuvant radiotherapy (RT) is administered to prevent ipsilateral breast tumor recurrence (IBTR), including a new ipsilateral cancer. The EBCTCG meta-analysis showed a majority of patients treated with surgery only to be recurrence free at 10 years, and more than 10% to suffer an IBTR despite RT, thus implying considerable over- and under treatment. A wide range of prognosticators, including multigene tests, are well established, but we lack predictive factors for RT, which is the aim in the present study.

Patients and methods: Fresh frozen tissue from 340 patients operated with BCS with or without RT and with or without IBTR was collected (without IBTR N=196, with IBTR n=144). Patients were stratified according to estrogen receptor (ER) status and RT, and divided into a training cohort (N=172) and a validation cohort (N=168). The training cohort was analyzed with whole transcriptome analysis (Illumina HT12 v4) and top discriminating genes for IBTR (N=155) were selected based on a random forest machine learning algorithm with recursive feature elimination and cross-validation. Further, genes described in the literature as associated with radioresistance were included in the panel to a total of 248 genes. A custom nCounter (Nanostring Technologies) gene expression panel was designed and both the training and validation cohorts were analyzed with the custom panel. Single-sample classifiers using a k-top scoring pairs algorithm were trained in the training cohort and validated in the validation cohort. Area under the curve (AUC) with a receiver operator characteristics (ROC) analysis were calculated and p-values were calculated with a log-rank test. All calculations were done using the R statistical environment.

Results: Our classifiers were prognostic for IBTR in the validation cohort among ER+ patients given RT (AUC 0.67, p=0.005), ER+ patients not given RT (AUC=0.89, p=0.015) and ER- patients given RT (AUC=0.78, p<0.001), while the number of ER- patients not given RT was too small for subgroup analysis (N=4). We also created a sequential algorithm were a first classifier was applied to test the risk of IBTR without RT. If low, the tumor was classified as “surgery only”. If classified as high, a second classifier was applied to test the risk of recurrence when given RT. If the risk was predicted low after RT, the tumor was classified as “radiosensitive”. If high, the tumor was classified as “radioresistant”. Among ER+ patients in the validation cohort, the “radiosensitive” tumors had an excellent effect of RT (p<0.001), the “radioresistant” had no effect of RT (p=0.4) and a very high risk of recurrence (55% at 10 years). The tumors predicted as “surgery only” had no effect of RT (p=0.4), and a lower risk of recurrence than the “radioresistant” patients (25% at 10 years).

Conclusions: Our targeted radiosensitivity gene expression panel could identify patients of high or low risk of LR, with or without RT. The most promising was however that it seems as the panel could be used as a predictive marker, i.e., finding patients that do, or do not, respond to RT. Further refinement and testing of the panel and models is ongoing.
Correlation of breast cancer index (BCI) predictive (HoxB13/IL17BR) results to nodal status and hormone receptor expression in early stage HR+ breast cancer

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Body: Background: The HoxB13/IL17BR (H/I) ratio, the endocrine response component of the Breast Cancer Index (BCI), was initially discovered in patients with LN- breast cancer, and validated as a predictive biomarker of benefit from extended endocrine therapy (EET) in a cohort from MA.17 that included both LN- and LN+ patients. Here, correlative analyses were performed to further characterize BCI Predictive (H/I) results with nodal status and quantitative hormone receptor expression.

Methods: Analyses were performed using data from the BCI Clinical Database for Correlative Studies, an IRB-approved de-identified database that contains >50 clinicopathologic and molecular variables from cases submitted for BCI in clinical practice (N=19,126). Clinicopathologic variables were abstracted from pathology reports, and were available for a subset of these cases. Cases from patients with confirmed nodal status were analyzed. Chi-squared tests and ANOVA were used to compare results between subgroups.

Results: Analyses included 13,114 patients (median age 58.9y; range 23-92y); 9562 were LN- and 3552 LN+. The distribution of individual H/I scores and proportion of patients classified as High H/I were generally similar in LN- and LN+ patients, though a modestly greater proportion of LN+ patients (46.2%) were classified as High H/I compared to LN- patients (42.0%; P<0.01). In both LN- and LN+ patients, median quantitative ER (qER) expression was slightly higher in patients with Low H/I than with High H/I; qPR showed the same trend but with a larger magnitude (P<0.0001 for both). Similar results were observed for percent positive staining by IHC (P<0.0001). In both LN- and LN+ patients, H/I showed a weak negative correlation with qER (LN-, 0.227; LN+, 0.192) and qPR expression (LN-, 0.311; LN+, 0.311).

Conclusion: In this study to evaluate potential biological correlates of BCI, results showed that H/I biomarker activity did not appear to be dependent on nodal status. Secondly, although ER expression is an established biomarker for endocrine sensitivity, High H/I status did not correlate with increased quantitative ER and PR expression. The H/I ratio may be an independent new marker for endocrine sensitivity independent of the strength of ER expression.
Title: Nomogram for predicting lymph node involvement in patients with invasive micropapillary carcinoma of breast: A SEER population-based study

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Body: Background: Invasive micropapillary carcinoma (IMPC) is an unusual and distinct subtype of invasive breast tumor with high propensity for regional lymph node metastases. Because of its lymphotropic nature at initial presentation, IMPC is considered to have an unfavorable prognosis when compared with invasive ductal carcinoma (IDC). The aim of this study was to identify risk factors accounting for its lymphotropic features and to develop a nomogram to predict the probability of lymph node involvement in IMPC.

Patients and Methods: A retrospective review of the clinical and pathology records was performed in patients diagnosed with IMPC between 2003 and 2014 from Surveillance, Epidemiology, and End Results (SEER) database. Training set comprised patients diagnosed between 2003 and 2009, while validation set included patients diagnosed thereafter. Ethical approval of the study was granted by the Institutional Review Board of Fudan University Shanghai Cancer Center. Multiple logistic regression analysis was conducted. A logistic regression model was used to construct the nomogram in the training set and then validated in the validation set. Nomogram performance was quantified with respect to discrimination and calibration.

Results: Overall, 1407 patients diagnosed with IMPC were enrolled, of which 527 in training set and 880 in validation set. The demographic characteristics were comparable within sets. Larger lesion, younger age at diagnosis, black ethnic and lack of hormone receptor expression were significantly related to regional nodes involvement. The AUC of the nomogram was 0.735 (95 percent confidence interval 0.692 to 0.777), demonstrating a good prediction performance. A calibration curve for the nomogram was plotted to evaluate the agreement between actual (observed) outcomes and expected probabilities. The slope of the calibration curve was close to 1, which indicated excellent calibration of the nomogram. The performance of the nomogram was further validated in the validation set, in which AUC was 0.734 (95 percent confidence interval 0.701 to 0.767).

Conclusions: The striking difference between IMPC and IDC remains the increased lymph node involvement in IMPC and therefore merits aggressive treatment. The nomogram based on the clinical parameters was established, which could accurately predict regional lymph node status. This nomogram would facilitate evaluating lymph node state preoperatively and thus treatment decision-making of individual patients, especially in neoadjuvant settings.
**2017 San Antonio Breast Cancer Symposium**

**Publication Number:** P4-09-11

**Title:** Computer extracted features of tumor grade from H&E images predict oncotype DX risk categories for early stage ER+ breast cancer

Jon R Whitney, David Romeo-Bucheli, Andrew Janowczyk, Shridar Ganesan, Michael Feldman, Hannah Gilmore and Anant Madabhushi. 1Case Western Reserve University, Cleveland, OH; 2Universidad Nacional de Columbia, Bogota, Colombia; 3Rutgers Cancer Institute, New Brunswick, NJ; 4University of Pennsylvania, Philadelphia, PA and 5University Hospitals Cleveland Medical Center, Cleveland, OH.

**Body:**

**Introduction:** The ODx test is a 21 gene assay that is currently employed for separating Estrogen Receptor positive (ER+) breast cancer patients into low (L) and high (H) risk of recurrence categories, helping clinicians decide if adjuvant chemotherapy is appropriate. In this study, we sought to explore whether computer extracted features pertaining to tumor grade (nuclear pleomorphism, tubule count, mitotic index) in conjunction with a machine learning classifier were predictive of the corresponding ODx risk category for ER+ breast cancer patients.

**Design:** First, 2000x2000 pixel sub-regions of digitized H&E slides at 40x are processed to both identify and segment epithelial and stromal nuclei using a combination of watershed and deep learning (DL). 247 nuclear features consisting of architecture, shape, and texture features were extracted from these segmentations. Subsequently, the mitotic and tubule related features were extracted at each nuclei candidate using DL detectors. The input to this process was a binary mask computed by thresholding a blue ratio transformed image using Otsu’s method. The identified regions were analyzed using DL to determine if a nucleus is a part of a tubule, and/or if it is mitotic. Finally, all of these features were combined, evaluated using Ranksum feature ranking, and then used to generate predictive models using four different supervised machine learning classifiers - random forest, support vector machine, linear discriminant analysis, and a neural network - via a 3-fold cross validation scheme. The classifiers were evaluated by their ability to distinguish between the four different classification tasks presented above using the area (AUC) under the Receiver Operating Characteristic (ROC) curve: 1) L ODx and L mBR grade vs. H ODx and H mBR grade (L-L vs. H-H), 2) L ODx vs. H ODx, 3) L ODx vs. Intermediate (T) and H ODx, 4) L and T ODx vs. H ODx.

**Results:** The highest performing features were consistently mitosis, epithelial architectural, and tubule features. Classification accuracy ranged from 0.61 (L vs. T and H) to 0.97 (L-L vs. H-H) (Table 1). These features were able to provide the highest level of classification utility for the most distinct cases (L-L vs. H-H) and had less classification accuracy with classification problems involving more difficult T cases.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Max AUC</th>
<th>Number top 10 Features in each category</th>
<th>Number top 10 Features in each category</th>
<th>Number top 10 Features in each category</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-L vs. H-H (N=36)</td>
<td>0.97</td>
<td>3</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>L vs. H (N=72)</td>
<td>0.77</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>L vs. T and H (N=125)</td>
<td>0.61</td>
<td>2</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>L and T vs. H (N=125)</td>
<td>0.75</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 1: Maximum AUC, and best features used to obtain those results.

**Conclusion:** Computer derived features pertaining to nuclear architecture and mitotic index were predictive of ODx risk categories. Additional independent validation of these findings is needed in a separate test set.
Body: Background: Ductal Carcinoma in Situ (DCIS) is a pre-invasive stage of breast cancer, where malignant cells line the duct but have not spread into other parts of the breast. Oncotype DX (ODX) is a genomic test, which divide patients into three risk of recurrence categories (Low, Intermediate, and High) to help physicians determine if patients require adjuvant therapy. However, ODX is expensive, tissue destructive, and has a turnaround time of 7-10 days. There has been an interest in the use of image analysis of routine H&E histopathology slides to predict the course of the disease; the rationale being that the analytics are able to unearth subtle sub-visual cues regarding disease morphology that may escape visual examination. In this work, we evaluate the role of computer extracted features of nuclear morphology and the necrotic regions from surgically resected specimens to predict ODX categories in patients with DCIS.

Methods: H&E slides from breast tissue of 37 patients who were diagnosed with DCIS and underwent a lumpectomy were acquired. Nine of the 37 had high ODX score (higher than 54), while the rest had a low score (lower than 39). All the slides were digitized on a Philips slide scanner. For each image, a watershed algorithm segmented the individual nuclei, which were used to generate 230 nuclei features including nuclear architecture, nuclear shape and nuclear texture features within each candidate breast duct. In addition, we captured the area of necrosis and empty lumen region inside breast ducts to generate features pertaining to tubule packing.

The average feature values for each patient were calculated across all the breast ducts in each slide. A 3-fold cross validation scheme with 50 repetitions was used with the Support Vector Machine (SVM) classifier to predict the ODX risk category for each patient. We used a covariance algorithm to select the top 4 features that were independent of each other but relevant to the ODX class label.

Results: The top ranked features included features from three categories: nuclei architectural features (standard deviation of triangle area in Delaunay graph, skewness of edge length in Cell Cluster Graph), nuclear texture (standard deviation of Haralick matrix intensity) possibly reflecting chromatin patterns in the cell, and the Tubule Packing Ratio, a measure of the ratio of necrosis area and empty lumen area inside the breast ducts compared to the whole breast duct area. The SVM in conjunction with these 4 features yielded a mean area under receive operator characteristic curve (AUC) of 0.95 in correctly predicting high and low ODX risk categories.

Conclusion: We found that our histomorphometry features pertaining to nuclear arrangement, nuclear texture and necrosis could differentiate between DCIS patients with high and low ODX risk categories. Additional independent validation of the approach is needed to confirm the preliminary findings presented here.
Title: A prognostic predictive model based on the correlation of standard clinicopathologic characteristics with oncotype Dx 21-gene recurrence score for node-negative and ER positive breast cancer

Michael R Mankbadi1, Yi Luo1, Madiha Yasin1, Bertha Ben Khalouq1 and Rebecca Moroose2. 1University of Central Florida College of Medicine, Orlando, FL and 2UF Health Cancer Center – Orlando Health, Orlando, FL.

Body: BACKGROUND: The Oncotype Dx Breast Cancer Assay (21-gene recurrence assay) is a prognostic tool that produces a recurrence score, which estimates the probability of distant recurrence within 10 years, given 5 years of adjuvant endocrine therapy as well as predicts benefit from adjuvant chemotherapy. The primary objective of this study was to explore if standard clinicopathologic variables independently correlate to the recurrence score. As a secondary objective, we explored if a model based on the clinicopathologic variables can accurately predict recurrence score.

METHODS: We conducted a retrospective chart review of 507 patients with node-negative, estrogen receptor (ER) positive breast cancer from the UF Health Cancer Center at Orlando Health. Of the 507 patients, 84 did not meet inclusion criteria (n = 423). The following factors were correlated with recurrence score (RS): age, tumor size, ER, PR, ki67, HER2 (IHC), HER2 (FISH), and tumor grade. Although the RS is a continuous variable, scores are also used to categorize patients into three risk groups: low (RS<18), intermediate (18≤RS<31), and high (RS≥31). This study evaluated variation of the HER2 levels within this subset of HER2 negative patients.

RESULTS: In univariate analysis, there were no significant correlations between age, ER, tumor size, Ki67 and RS. There were statistically significant correlations between PR (r=-.42, p<.001), HER2 (IHC) (r=.097, p<.05), HER2 (FISH) (r=0.40, p<.001), and tumor grade (r=0.37, p<.001) and RS. As a second step, we used these results and clinical expertise to guide a predictive model. As expected, independently, PR and tumor grade were significant predictors of RS (βs=-.096, .37, respectively; p<.05). However, HER2 (IHC) was not, β=.097, p =.054. As a final step, three pathologic factors (PR, HER2 by IHC, and tumor grade) were used to predict recurrence score. In the multivariate analysis, we found a statistically significant model, (R = 0.54, R_adj = .30; F (3, 399) = 57.17, p <.001), where, PR (β= -.096), HER2 (IHC) (β= 2.007), and tumor grade (β= 4.778). Concordance with the 21-gene assay was as follows: 76.0%, 54.5%, and 66.7% for low, intermediate, and high-risk groups, respectively. As clinical guidelines are not firmly established for the use of chemotherapy in the intermediate risk group it is also valuable to estimate the two-step discordance, an estimated RS that is high, when the 21-Gene recurrence score is low and vice versa. The two-step discordance was 2.2% and 0.0% for the low and high risk groups, respectively.

CONCLUSION: The data presented here suggests that it may be possible to create a model based on standard clinicopathologic parameters to predict the 21-gene recurrence score in node-negative, ER positive breast cancer patients. In practice, a 21-gene recurrence score assay is not always possible. We present an option to identify patients whose risk category can be confidently determined from the standard clinicopathologic variables alone, thereby reducing medical cost. Admittedly, this model has not undergone validation nonetheless, the data suggest the potential for a novel; low-cost; high reach diagnostic tool.
Title: Analysis of breast cancer recurrence using gene set enrichment analysis

Anupama Praveen Kumar\(^1\), Albert J Kovatich\(^2\), Angélique Biancotto\(^6\), Foo Cheung\(^3\), Jan K Davidson-Moncada\(^4\), Leonid Kvecher\(^1\), Jianfang Liu\(^1\), Yuanbin Ru\(^1\), Audrey W Kovatich\(^3\), Brenda Deyarmin\(^1\), Jamie Leigh Fantacone-Campbell\(^2\), Jeffrey A Hooke\(^2\), Praveen Kumar Raj Kumar\(^1\), Hallgeir Rui\(^6\), Hai Hu\(^1\) and Craig D Shriver\(^7\). \(^1\)Chan Soon-Shiong Institute of Molecular Medicine at Windber, Windber, PA; \(^2\)Clinical Breast Care Project, Murtha Cancer Center, Uniformed Services University / Walter Reed National Military Medical Center, Bethesda, MD; \(^3\)National Institutes of Health, Bethesda, MD; \(^4\)MacroGenics, Inc, Rockville, MD; \(^5\)MDR Global Systems, Windber, PA; \(^6\)Medical College of Wisconsin, Milwaukee, WI and \(^7\)Murtha Cancer Center, Uniformed Services University / Walter Reed National Military Medical Center, Bethesda, MD.

Body: Background: Even after successful treatment of primary breast tumors, there is a continued risk of recurrence. The risk varies between subtypes and there are ongoing efforts that aim to improve prediction of such risks for individual patients. Detection of subclinical metastases might be achieved by biomarkers in blood. In this study, we profiled protein expression in blood plasma from patients with known clinical outcome (recurrence vs no recurrence) to identify prognostic markers of breast cancer recurrence.

Methods: The subjects and specimens were made available through the Clinical Breast Care Project using IRB-approved protocols. We analyzed blood plasma samples taken at the time of diagnosis from consented patients who subsequently relapsed (33 cases) as well as those with no disease recurrence (31 controls). Based on hormone receptor and lymph node status the samples were grouped as: \(\text{ER}^-/\text{HER2}^-\) (17 cases/15 controls), \(\text{ER}^+/\text{LN}^+\) (10/10) and \(\text{ER}^+/\text{LN}^-\) (6/6). We used aptamer-based SOMAscan assay platform to study the expression of 1252 proteins. We analyzed the protein expression data by using their coding genes in order to apply the Gene Set Enrichment Analysis method (GSEA v.2, Broad Institute). Pathway databases of KEGG, REACTOME, BIOCARTA and C4 collection were used. Significant gene sets were called at 5% FDR, and overlaps and low coverage gene sets (Tags <70%) were removed. Statistical analysis and clustering were done using R.

Results: Unsupervised clustering showed some difference in signal in the \(\text{ER}^+/\text{LN}^-\) group. Even though there was a lack of significantly differentiated proteins between the cases and controls of this group, many significant gene sets were identified. After applying the cutoff filters and removing the overlaps, there were 5 gene sets enriched with the pathway collection, involved in B-cell receptor signaling, mRNA metabolism, tight junction and SCF-KIT signaling. Similarly, 9 gene sets from the MORF compendium were differentially expressed with the C4 collection and included neighborhood genes of NME2, ACTG1, EIF3S2, AP2M1, DAP3, UBE2I, NPM1, AATF and NPM1. In contrast, neither differentially expressed proteins nor gene sets were identified from the \(\text{ER}^+/\text{LN}^+\) and \(\text{ER}^-/\text{HER2}^-\) groups. Since the sample size of the \(\text{ER}^+/\text{LN}^-\) group was small, we conducted a similar analysis by randomly choosing 6 case and control samples in the other two groups respectively. There were still no differentially expressed proteins or gene sets identified above the specified cutoff parameters.

Conclusion: Using plasma protein expression data we identified underlying gene sets differentially expressed between \(\text{ER}^+/\text{LN}^-\) patients who had cancer recurrence and no recurrence. Many genes in these sets were already known biomarkers (e.g. PTEN, AKT1, STAT3, SET etc.). These results can be used for understanding patterns of recurrence in different cancer subtypes. Further research is needed to estimate the clinical significance of these gene products.

The views expressed in this article are those of the author and do not reflect the official policy of the Department of Army/Navy/Air Force, the Department of Defense, or U.S. Government.
Title: Real-world clinical outcomes in BRCA-positive metastatic breast cancer patients treated in the community oncology setting

Arthur C Houts¹, Temitope O Olufade², Rahul Shenolikar² and Mark S Walker¹. ¹Vector Oncology, Memphis, TN and ²AstraZeneca, Gaithersburg, MD.

Body: Background: BRCA-mutated breast cancer remains a heterogeneous disease, with variations in histology, response to treatment, and survival outcomes. These cancers are more likely to be negative for estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 (HER2) or triple negative (TN). However, some are hormone receptor-positive (HR+) and amenable to hormone treatment. We compared treatment patterns and clinical outcomes in patients with BRCA-mutated metastatic breast cancer (MBC) who were either TN or HR+/HER2-negative using real-world data.

Methods: This retrospective study examined progression-free survival (PFS) and overall survival (OS) in a US community oncology sample of adult patients with BRCA-mutated MBC for up to three lines of treatment. Medical records from the Vector Oncology Data Warehouse were used. Disease progression was determined from chart review. Overall survival was determined from start of first-line (L1) treatment; patients without evidence of death were censored at the last observed visit. Cox models of PFS and OS were adjusted for age, race, performance status, tumor grade, and bone metastasis.

Results: The study included 57 TN patients and 57 HR+ patients with BRCA mutation. BRCA 1 and BRCA 2 mutations were more frequent in patients with TN disease and HR+ disease, respectively. In TN disease, the most common L1 treatments, n (%), were bevacizumab 10 (20.4%), capecitabine 10 (20.4%), carboplatin/gemcitabine 8 (16.3%), carboplatin plus other 7 (14.3%), and paclitaxel 5 (10.2%). In HR+ patients, L1 treatments included aromatase inhibitors 14 (24.6%), fulvestrant 10 (17.5%), tamoxifen 7 (12.3%), and capecitabine 5 (8.8%). The between-group difference in PFS following L1 was statistically significant favoring HR+ disease (see Table), but not following lines 2 or 3. Overall survival was also significantly longer for the HR+ group (see Table). Presence of bone metastasis was a significant predictor of higher risk for disease progression.

Conclusions: In this real-world sample of patients with BRCA1- or BRCA2-mutated MBC, those with TN disease had significantly worse outcomes after L1 (PFS, OS) compared with similar patients with HR+ disease. Triple-negative disease was associated with poorer prognosis, demonstrating a need for new treatment options for patients with BRCA-mutated TN MBC.

Baseline Characteristics and Outcomes

<table>
<thead>
<tr>
<th></th>
<th>TN N=57</th>
<th>HR+ N=57</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median yrs</td>
<td>46</td>
<td>51</td>
</tr>
<tr>
<td>Performance Status Impaired, n (%)</td>
<td>2 (3.5)</td>
<td>4 (7.0)</td>
</tr>
<tr>
<td>Any CCIa Comorbidity, n (%)</td>
<td>18 (31.6)</td>
<td>11 (19.3)</td>
</tr>
<tr>
<td>BRCA1, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>40 (70.2)</td>
<td>18 (31.6)b</td>
</tr>
<tr>
<td>-</td>
<td>17 (29.8)</td>
<td>39 (68.4)b</td>
</tr>
<tr>
<td>BRCA2, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>16 (28.1)</td>
<td>39 (68.4)b</td>
</tr>
<tr>
<td>-</td>
<td>41 (71.9)</td>
<td>18 (31.6)b</td>
</tr>
<tr>
<td>PFS L1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median Months (95% CI)</td>
<td>6.13 (4.2, 9.4)</td>
<td>12.09 (7.1, 14.5)</td>
</tr>
<tr>
<td>Adj PFS HR (95% CI) for TN vs HR+</td>
<td>1.714 (1.082, 2.717)</td>
<td>P=.022</td>
</tr>
<tr>
<td>Bone Metastasis HR (95% CI) Present vs Absent</td>
<td>1.631 (1.039, 2.561)</td>
<td>P=.033</td>
</tr>
</tbody>
</table>
OS from Start of L1

<p>| | | |</p>
<table>
<thead>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Median Months (95% CI)</td>
<td>23.43 (15.4, 26.4)</td>
<td>38.41 (28.9, 67.4)</td>
</tr>
<tr>
<td>Adj OS HR (95% CI) for TN vs HR+</td>
<td>1.869 (1.072, 3.256)</td>
<td>P = .027</td>
</tr>
<tr>
<td>HR (95% CI) for Race Minority/Unknown vs White</td>
<td>2.040 (1.118, 3.723)</td>
<td>P = .020</td>
</tr>
<tr>
<td>HR (95% CI) for Tumor G3 vs Other</td>
<td>3.010 (1.299, 6.973)</td>
<td>P = .010</td>
</tr>
</tbody>
</table>

a Charlson Comorbidity Index

b P < .001
2017 San Antonio Breast Cancer Symposium

Publication Number: P4-10-01

Title: Race, place and delays in breast cancer treatment across the care continuum in the Carolina breast cancer study

Katherine E Reeder-Hayes\textsuperscript{1,2}, Sophie E Mayer\textsuperscript{3}, Andrew Olshan\textsuperscript{2,3}, Lisa A Carey\textsuperscript{1,2}, Stephanie B Wheeler\textsuperscript{2,4}, Chiu-Kit Tse\textsuperscript{2} and Melissa Troester\textsuperscript{2,3}. \textsuperscript{1}University of North Carolina; \textsuperscript{2}University of North Carolina Lineberger Comphrehensive Cancer Center; \textsuperscript{3}University of North Carolina and \textsuperscript{4}University of North Carolina.

Body: Black women have a persistent disparity in breast cancer survival after controlling for clinical factors, suggesting that differential receipt or efficacy of treatment may contribute to poorer outcomes. Delays in initiating treatment and subsequent spillover effects into time to completion are one proposed mechanism by which these survival disparities may occur. The Carolina Breast Cancer Study Phase III is a large population-based study of North Carolina women with incident breast cancer. For this analysis, we included black (n=1328) and white (n=1331) women with stage I-III disease whose treatment included surgery with or without adjuvant therapies. Patients were grouped by treatment pathway (surgery only, surgery + chemotherapy, surgery + radiation, or all three modalities). We investigated the association of race with delays in treatment initiation, duration and completion. Delays in initiation of first treatment were modeled in two ways: i) time to initiation >30 days from diagnosis, and ii) time to initiation above the 75\textsuperscript{th} percentile. Extended duration and delay in completion were defined as being > 75\textsuperscript{th} percentile in days between initiation and end of treatment (duration) or days from diagnosis to end of treatment (completion) compared to others in the same pathway. Models controlled for treatment pathway, age, and tumor characteristics. Additional models controlled for demographic factors (marital status, income, education, insurance) and county-level factors related to care access (% urban and health professional shortage area status).

In analyses adjusted only for age at diagnosis, black women had a significantly higher risk of treatment delays whether measured by delay>30 days (risk ratio (RR) 1.24, 95% CI 1.12-1.37) and or by highest quartile of time to initiation (RR 1.23, 95% CI 1.07-1.40) compared to whites. They were also at higher risk of extended treatment duration (RR 1.47, 95% CI 1.29-1.69) and time to completion (RR 1.58, 95% CI 1.37-1.81) compared to others in the same pathway. Interestingly, adjustment for tumor characteristics did not impact effect estimates. While adjustment for demographic factors had little impact on the association of race with delays in initiation, it attenuated the association of race with delays in treatment duration and completion. Further adjustment for care access factors slightly attenuated the association of race with treatment initiation and completion, but did not impact associations with treatment duration. Significant racial disparities remained in delay across all phases of care after adjustment for clinical, demographic and access factors.

Overall, black women were at higher risk of delays in treatment initiation, extended duration and time to completion than white women receiving similar treatment, and these disparities appear to be compounded over the care continuum. These findings suggest that racial differences in income, education and insurance may partly explain observed disparities in treatment duration and time to completion, whereas care access factors may have more impact on disparities in treatment initiation, and interventions that target both patient-level and care access barriers may be needed to improve timely delivery across the care continuum.
Title: Validation of a clinical informatics system for online multidisciplinary expert opinions: Mapping treatment recommendations to the NCCN resource-Stratified framework

Rajendra A Badwe¹, Sudeep Gupta², Nancy Feldman³, CS Pramesh⁴, Naresh Ramarajan⁵, Gitika Srivastava⁶, Nita Nair⁷ and Benjamin O Anderson⁸. ¹Breast Disease Management Group, Tata Memorial Centre, Mumbai, India; ²Breast Disease Management Group, Tata Memorial Centre, Mumbai, India; ³University of California Los Angeles- Olive View Medical Center (UCLA-OVMC), Los Angeles, CA; ⁴National Cancer Grid, India and ⁵National Comprehensive Cancer Network.

Body: Background: Most cancer patients in Low and Middle Income Countries (LMIC) cannot afford effective, expensive, evidence based therapies. Therefore, oncologists must tailor treatment plans to individual resource constraints. To support this, NCCN has created a Resource-Stratified Framework® (NCCN-RSF), which is an evidence-based four-tier prioritization scheme. Further, only a fraction of patients in LMIC have ready access to oncologists. In India, there are only ~1600 oncologists for 1.8 million patients. To bridge this gap, Navya's clinical informatics based mobile ExpertApp combines learning from evidence, prior tumor board decisions, patient resource constraints, and quick review from TMC NCG oncologists to recommend tailored treatment plans to patients via an online expert opinion service. 11865 patients in 22 LMIC have reached out to receive an online expert opinion through Navya (ASCO 2017). This study maps Navya to NCCN-RSF as an evidence-based index for resource-sensitive treatment selection.

Methods: All breast cancer patients who received an online expert opinion from TMC NCG Navya between July 1st 2014 and April 30th 2017 were included. Navya systematically gathered information on patient resource constraints (such as affordability for Trastuzumab). Navya recommendations (breast and nodal surgery, radiation site and fractionation, drug and dose density etc.) were mapped to NCCN-RSF resource tiers (Basic, Core, Enhanced, Parent guideline). Reasons were categorized for Navya recommendations not present in NCCN-RSF.

Results: 616 patients (36.3% metastatic), mostly from India, received 1203 recommendations. At the specific treatment protocol level, 88.3% of Navya recommendations mapped with at least one NCCN-RSF resource tier (Table 1). 78.5% mapped to the Enhanced tier. Only 8.6% of recommendations mapped to Parent guidelines, and did not require tailoring for resource constraints. Fewer than 2% recommendations mapped to Core and none to Basic. 11.7% recommendations were not present in NCCN-RSF, for minor reasons such as substitution of a drug within the same class (35.8%) (e.g., Epirubicin for Adriamycin), dose dense protocols (14.3%) (e.g., 3 weekly Paclitaxel vs weekly Paclitaxel), and recommending Trastuzumab for less than a year for patients unable to afford year long therapy (14.3%), currently not included in NCCN-RSF.

Table 1 - Mapping Navya to NCCN RSF

<table>
<thead>
<tr>
<th>NCCN RSF Tiers</th>
<th>HIGH LEVEL: Multimodality treatment and sequencing (1203)</th>
<th>INTERMEDIATE: Within modality treatment categories (1188)</th>
<th>GRANULAR: Specific treatment protocols (1140)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.g. Neoadjuvant vs Adjuvant Chemo</td>
<td>Anthracycline vs Taxane</td>
<td>Hypofractionation vs Standard XRT</td>
<td></td>
</tr>
<tr>
<td>At least one Tier</td>
<td>98.8%±0.6</td>
<td>96%±1.1</td>
<td>88.3%±2</td>
</tr>
<tr>
<td>Enhanced</td>
<td>94.4%±1.3</td>
<td>91%±1.7</td>
<td>78.5%±2.7</td>
</tr>
<tr>
<td>Core</td>
<td>1.9%±5.6</td>
<td>1.2%±5.7</td>
<td>1.2%±5.8</td>
</tr>
<tr>
<td>Parent NCCN</td>
<td>2.4%±5.6</td>
<td>3.8%±5.6</td>
<td>8.6%±5.5</td>
</tr>
</tbody>
</table>

Conclusion: Navya's treatment recommendations are sensitive to resource constraints and map to peer reviewed and evidence based NCCN RSF, primarily at the Enhanced tier. Navya's clinical informatics based online service scales access to resource...
constrained treatment selection for large numbers of patients in LMIC without easy access to oncologists.
Title: Racial variation in effects of gene expression profiling on chemotherapy use

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Body: Gene expression profile (GEP) testing in early hormone receptor-positive (HR+) breast cancer can inform adjuvant treatment decisions by providing refined estimates of prognosis and chemotherapy benefit. GEPs may also downshift chemotherapy use, potentially avoiding excess morbidity. Multiple studies have reported racial disparities in access to GEP testing. In this study, we examine the effect of race and GEP testing on chemotherapy utilization in early stage HR+ breast cancer.

From The University of North Carolina Cancer Information and Population Health Resource (CIPHR), a statewide linkage of cancer registry and administrative claims data, we studied all women eligible for GEP testing, including those with HR+, T1-2, N0 or N1 unilateral primary breast cancers diagnosed from 2005 to 2012. Separate analyses were performed for N0 and N1 patients. Patients were required to have breast surgery within 6 months of diagnosis and no chemotherapy prior to surgery. Use of GEP testing and chemotherapy treatment were defined from insurance claims, while race was defined using cancer registry data. Propensity score adjustment by standardized mortality ratio weighting (SMRW) was used to control for differences in measured patient characteristics between treatment groups in a non-randomized cohort. Propensity score models included age, race, diagnosis year, co-morbidity, and tumor features. Propensity weighted Poisson models were then used to calculate the adjusted risk of receiving chemotherapy. To investigate differential effects of covariates on chemotherapy across racial groups, we used race-stratified models in the N0 cohort. Due to small sample sizes, race-stratified models were not used for N1 patients.

The cohort included 11,958 women of whom 84.5% were non-Hispanic white (NHW), 12.9% black, and 2.6% other race/ethnicity. For stratified analyses, race was dichotomized to NHW versus non-white. In the N0 cohort (n=9,671), 11.5% of untested NHW women, 20.8% of tested NHW, 16.8% of untested non-whites, and 21.9% of tested non-whites received chemotherapy. In SMRW-adjusted models, GEP testing was associated with downshifts in chemotherapy for N0 patients (RR 0.79, 95% CI 0.72-0.86) and N1 patients (RR 0.46, 95% CI 0.40 - 0.54). In race-stratified analyses of N0 patients, use of GEP testing was associated with a decrease in chemotherapy of 19% among NHW women (RR 0.81, 95% CI 0.72 - 0.91) and 25% among non-white women (RR 0.75, 95% CI 0.57 - 0.99). Taxane-based regimens were most common, but a substantial portion of chemotherapy-treated patients (43%) received anthracycline-based regimens with or without a taxane.

We conclude that GEP testing was associated with decreases in chemotherapy use after propensity adjustment for patient and tumor factors, with greater reductions among non-white patients and node-positive patients. Possible explanations include over-treatment of black women in the absence of testing due to higher perceived risk, racial differences in provider recommendation or chemotherapy choice among tested women where benefit is uncertain, such as for intermediate recurrence scores. Further studies are underway to evaluate racial differences by recurrence score group and the effect of GEPs on racial disparities in outcome.
Title: EMBRACE (Ending metastatic breast cancer for everyone): A comprehensive approach to improve the care of patients with metastatic breast cancer

Melissa E Hughes¹, Elizabeth S Frank¹, Margaret S Merrill¹, Rebecca A Santiago¹, Nicole Kuhnly¹, Lindsey M Crowley¹, Gunjan Gupta¹, Eric P Winer¹ and Nancy U Lin¹. ¹Dana-Farber Cancer Institute, Boston, MA.

Body: Background: In contrast to early stage breast cancer, the quality of care for patients with metastatic breast cancer (MBC) has been relatively understudied, as have interventions to improve care in the real-world setting. Patients with MBC face a variety of unique needs related to their disease, treatment options, and supportive care. Little attention has been focused on leveraging the strengths of academic and community-based settings to provide optimal care for these patients. To address these critical issues, we have designed and implemented a comprehensive program that combines clinical care, clinical research, physician engagement and patient education to optimize the care of MBC patients.

Methods: We developed a consistent and comprehensive intake process and follow-up approach for MBC patients who were seen at least once in the Breast Oncology Clinic (BOC) at Dana-Farber Cancer Institute (DFCI). A key component of our approach is the EMBRACE coordinator who meets with each MBC patient at the first clinic visit to review the clinical program, available educational and supportive resources, and consents to research studies. Each coordinator supports the DFCI-based oncologist and follows a discrete patient panel longitudinally, for whom they are responsible for facilitating referrals to supportive care resources, identifying potential candidates for trial prescreening, tracking availability of results from molecular testing for clinical trial matching, facilitating communication between DFCI-based providers and referring providers and organizing re-consultation visits when clinically appropriate. The coordinator contacts patients every 3 months to inquire about the patient's overall health and needs and provides updates on upcoming educational and supportive care activities at our institution.

Educational offerings have been expanded to include a bi-annual newsletter, quarterly email updates, webcasts and an annual educational patient forum. Results: The program was fully implemented in the BOC across 27 oncologists in August 2016, after the start of a pilot in July 2015. On average, the program enrolls 30 to 40 new MBC patients per month at their initial visit. The EMBRACE coordinators currently support the DFCI-based oncologists in the care of approximately 1500 new and existing MBC patients and facilitate collaborations with 350 referring providers.

Conclusions: The EMBRACE program has made a tangible improvement in the quality of care for patients with MBC in our clinic. We have successfully established the infrastructure of the coordinator role and a robust tracking system to support the patient, DFCI-based provider, and referring provider. While the program has been solely based at DFCI, we believe that our approach has the potential for impact beyond our institution and ultimately serve as a model for enhanced academic-community-patient partnership.
Title: Engaging linguistically and ethnically diverse low income women in health research: A randomized controlled trial

Galen Joseph¹, Alyssa Nickell², Elly Cohen⁵, Nancy J Burke⁴, Susan Colen⁴, Katie Lawlor², Claudia Guerra¹ and Susan L Stewart⁴. ¹University of California, San Francisco, San Francisco, CA; ²The Shanti Project, San Francisco, CA; ³University of California, Merced, Merced, CA; ⁴University of California, Davis, Davis, CA and ⁵BreastCancerTrials.org, San Francisco, CA.

Body: Background: Underserved breast cancer survivors are typically offered fewer opportunities to participate in cancer research. To address this disparity, a community based navigator program, Shanti's Margot Murphy Breast Cancer Program (Shanti) initiated a collaboration with UCSF researchers and BreastCancerTrials.org (BCT), a nonprofit clinical trials matching service to explore the potential role of a trusted community-based organization as a source of culturally appropriate education and access to clinical trial information. Through formative research, we developed the Health Research Engagement Intervention (HREI), a one-on-one navigator-client education session emphasizing the range of treatment and non-treatment quality-of-life and observational studies, conducted at a time when the participant is not in the initial crisis of diagnosis. The HREI ends by providing participants with an information card listing BCT and other organizations that provide information about health research for breast cancer patients and survivors.

Methods: We tested the HREI in a randomized controlled trial, comparing the HREI to simply providing the information card. Pre and post intervention surveys one month apart measured our primary outcome of health research information-seeking behavior. Secondary outcomes include health research knowledge, attitudes towards research participation, and health empowerment. All Shanti clients who spoke English, Cantonese or Spanish and had “low care navigation needs” (either completed treatment or no longer in the crisis of initial diagnosis and/or burdened by treatment protocols) were eligible.

Results: We recruited 133 Shanti Clients, including 59 who spoke English, 48 Cantonese, and 26 Spanish; 66 were randomized to the intervention arm and 67 to the control arm, and 130 completed both pre- and post-test surveys. Almost one-third of participants in both the intervention and control arms reported having talked to someone about health research or having called a telephone number or visited a website listed on the card (30% vs. 30%, p=0.94); a smaller proportion of participants confirmed that their information-seeking was related to the content of the educational materials (17% vs. 9%, p=0.22). On average the change from pre- to post-test in a 5-item knowledge score, adjusted for pre-test knowledge, was greater in the intervention group than in the control group (p=0.028), but the proportion of participants who were very confident that they could find health research information (had health empowerment) remained essentially unchanged in both study arms (intervention: 20% post vs. 21% pre, p=0.76; control: 25% post vs. 25% pre, p=1.00). Women were more likely to seek information if they had higher pre-test knowledge scores (odds ratio [OR]=3.5 per item, 95% confidence interval [CI] 1.5-8.4) or a greater increase in knowledge from pre- to post-test (OR=2.2 per item, 95% CI 1.1-4.7); there was no association between information-seeking and health empowerment (OR=0.6, 95% CI 0.2-2.5) or study arm (OR=1.6, 95% CI 0.5-4.9).

Conclusion: The HREI had a positive impact on knowledge of health research but did not significantly affect health empowerment or health research information-seeking behavior.
Body: Background: The impact of age at diagnosis on clinical presentation and treatment delivery for triple negative breast cancer (TNBC) is unclear. Utilizing data from a prospective registry, the aim of this study was to further elucidate the age-dependent correlation between TNBC clinical-pathological features, and the implications of age-bias on treatment delivery and prognosis.

Methods: 480 subjects with stage I-III TNBC were enrolled in an IRB approved multisite prospective registry between 2011 and 2016. Clinical, demographic, treatment information was collected and patients were followed for recurrence and survival. Patients were categorized as older (>60 years) or younger groups (<60 years). Recurrence free survival (RFS) and overall survival (OS) were estimated according to the Kaplan-Meier method and compared among groups by log-rank test.

Results: 145 (30%) of 480 TNBC patients were older (> 60 years) at time of diagnosis. Compared to younger patients, older patients were more likely to present with screen detected vs symptomatic cancer (47% vs 25% p=<0.001), more likely to have node negative cancer (71% vs 61% p=0.030), stage I disease (42% vs 28% p=0.003), and low level (1-10%) ER or PR positivity (19% vs 12% p=0.046). Compared to the younger patients, older patients were less likely to have a BRCA1/2 mutation (6% vs 23% p=0.002) but more likely to have a prior history of hormone positive breast cancer (7% vs 1% p=0.0002). Compared to younger counterparts, older patients were less likely to receive neo/adjuvant chemotherapy (93% vs 99% p=0.0006), and less likely to receive > 4 cycles of neo/adjuvant chemotherapy (61% vs 78%, p=0.0003). Three year RFS for the entire cohort was 80% and was identical for older and younger patients at 80%. Three year OS for the entire cohort was 87% and was similar for older and younger patients. On multivariable analysis only tumor size and nodal status significantly impacted RFS.

Conclusions: A significant fraction (30%) of TNBC patients are older (> 60 years) at time of diagnosis. Despite presenting a with more favorable disease stage, older TNBC patients did not demonstrate better outcomes compared to the higher risk younger patients. The underlying reasons for this observation may be tumor biology differences between older and younger TNBC patients or perhaps could be related to underutilization of appropriate systemic chemotherapy (39% of older patients received ≤ 4 cycles of chemotherapy). Further studies are warranted on this subject.
Title: Patient-centered initiatives for improving trial participation of diverse patient populations in the open-label phase 3b compLEEment-1 study of ribociclib plus letrozole in the treatment of HR+/HER2- advanced breast cancer

Tania Small¹, Jaclyn Marsano-Feeley¹, Alberto Fernandez¹, Tina Grasso¹, Katherine Feldman¹, Stephanie Petrone¹, Najla Bugazia¹, Celena Wong¹, Julie Meyer¹, Katie Schutta¹, Das Purkayastha¹, Krystal Saintil¹, Elyse Spatz Caplan¹ and Katherine Waltman-Johnson¹. ¹Novartis Pharmaceuticals Corporation, East Hanover, NJ.

Body: Background: Reported 10-year survival rates in Caucasian, black, and Hispanic women with breast cancer are 80%, 66%, and 78%, respectively. One barrier to understanding disparities in these survival rates is the lack of data due to underrepresentation in clinical trials (including black, Hispanic, Native American, and Pacific Islander populations). US physicians at sites that treat minority patients with breast cancer identified lack of access to and financial burden of clinical trials as 2 key barriers to enrollment. Insufficient knowledge of genetic mutations specific to breast cancer is also a key barrier to identifying ideal treatment to extend survival in minorities. Patient-specific initiatives for the CompLEEment-1 study (CLEE011A2404; NCT02941926), a single-arm, international Phase 3b study of ribociclib (600 mg/d, 3 weeks on/1 week off) plus letrozole (2.5 mg/d) in adults with hormone receptor–positive, human epidermal growth factor–negative advanced breast cancer, were developed in the United States to increase the enrollment of diverse populations and better understand the disease and patient response to treatment.

Methods: The enrollment initiatives included formation of a board of leading physicians and academics to advise on awareness initiatives and the cultural competency of tools used in clinical trials, a site selection process that brought the trial to the patient's clinic, a simplified expense reimbursement program to reduce patient economic burden, and partnerships with the Bridge Group and the National Black Church Initiative to bring clinical trial awareness and education to the African American church community. A tumor collection companion study (NCT03050398) was created to identify mechanisms of resistance among racial and ethnic groups. These initiatives aimed to increase the percentage of minorities from previous trials (eg, 5.7% in the MONALEESA-2 trial) and identify potential biological differences associated with race or ethnicity that might affect treatment response.

Results: Enrollment in the CompLEEment-1 study is ongoing. Among the first 57 US patients enrolled, 8 (14.0%) were minorities (not identified as Caucasian or Asian), 32 (56.1%) were <65 years old, 7 (12.3%) were premenopausal women, and 2 (3.5%) were men. Thirty-one patients were enrolled in the simplified expense reimbursement program. Of the open study sites, 35 (52.2%) were located >30 miles outside of metropolitan areas. Of the open study sites with enrolled patients, 16 enrolled 1, 8 enrolled 2, and 5 enrolled ≥3 patients.

Conclusions: Following discussions with physicians and assessments of patient feedback to identify reasons for underrepresentation of minority patient populations in clinical trials, patient-centered initiatives were developed for the CompLEEment-1 study in the United States to simultaneously reduce barriers to trial participation, reduce economic burden of enrolled patients, and identify biomarkers of therapeutic sensitivity and resistance. These initiatives resulted in an initial increase in the percentage of minorities enrolled to better reflect real-world populations.
Title: Delay in diagnosis of breast cancer in Mexican young women: Report of the “Joven y Fuerte” prospective cohort pilot phase

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Body: Background: Delay in diagnosis and treatment initiation of breast cancer (BC) has been associated with advanced stages and poor outcome. In developed countries, age has not been solely reported as an independent predictor of diagnosis delay. In Mexico, median time since tumor detection to treatment initiation is about 7 months, but young women are underrepresented in these studies. We aim to describe time intervals related to diagnosis in Mexican young women with BC (YWBC).

Methods: Newly diagnosed YWBC were invited to participate as part of this prospective cohort. Patient accrual began in November 2014 at two public cancer centers in Mexico. Patients completed self-report surveys including questions regarding mode of detection, time from first symptom to medical appointment (patient interval) and time from first symptom to diagnosis (total interval). Pearson chi-square tests were used to examine the effects of patient and clinical characteristics on patient interval and clinical stage.

Results: 96 YWBC with median age at diagnosis of 35 y (range 21-40) were enrolled in our pilot phase. 82.3% had tumor detected by self or partner. 62.5% of YWBC were diagnosed as locally advanced disease (IIB-IIIC). Median tumor size was 3.5 cm (0.5-12.0), with node involvement in 66.7%. 53.1% of YWBC had a patient interval of <6 months, but roughly 27.1% had a total interval <6 months. While only 13.5% had a patient interval >12 months, 39.6% reached a total interval >12 months. Patient interval and clinical stage were not significantly associated with occupation, education, marital status, current partner or method of detection.

<table>
<thead>
<tr>
<th>Time</th>
<th>Patient interval</th>
<th>Total interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1 month</td>
<td>29 (30.2)</td>
<td>7 (7.3)</td>
</tr>
<tr>
<td>1-3 months</td>
<td>18 (18.8)</td>
<td>9 (9.4)</td>
</tr>
<tr>
<td>4-6 months</td>
<td>4 (4.2)</td>
<td>10 (10.4)</td>
</tr>
<tr>
<td>7-12 months</td>
<td>10 (10.4)</td>
<td>24 (25.0)</td>
</tr>
<tr>
<td>&gt;12 months</td>
<td>13 (13.5)</td>
<td>38 (39.6)</td>
</tr>
<tr>
<td>No symptoms</td>
<td>0 (0.0)</td>
<td>3 (3.1)</td>
</tr>
<tr>
<td>NA</td>
<td>22 (22.9)</td>
<td>5 (5.2)</td>
</tr>
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</table>

Method of Detection

<table>
<thead>
<tr>
<th>Method of Detection</th>
<th>N(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient/Partner detected tumor</td>
<td>79 (82.3)</td>
</tr>
<tr>
<td>Clinical detection</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Image detected</td>
<td>9 (94)</td>
</tr>
<tr>
<td>NA</td>
<td>8 (8.3)</td>
</tr>
</tbody>
</table>

Clinical stage

<table>
<thead>
<tr>
<th>Clinical stage</th>
<th>N(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2 (2.1)</td>
</tr>
<tr>
<td>IA</td>
<td>13 (13.5)</td>
</tr>
</tbody>
</table>
Conclusions: In this cohort, most patients had a greater total delay than previously reported in Mexico, possibly attributed to long health-system intervals, which could contribute to worse outcomes in YWBC. The prospective nature of this study allows the recollection of biologic characteristics, treatment scheme and adherence to treatment, to determine their impact on clinical outcome besides diagnosis delay. “Joven & Fuerte”, the first dedicated program for the care of young breast cancer patients in Latin America, aims to develop YWBC-tailored interventions to early diagnose or “downstage” BC among young women by endorsing patient navigation, increasing general population awareness and improving providers' knowledge in low-middle income countries, such as Mexico.
An active approach to genetic counseling in Trinidadian women with breast cancer

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Introduction

Breast cancer (BC) is the leading cause of cancer death in Caribbean women. The prevalence of deleterious mutations in BRCA1/2, PALB2 and RAD51C among unselected BC patients in the Caribbean is 5-25%. We previously reported data on low usage of genetic counseling services by Bahamian women. In order to improve the dissemination of genetic testing results to Caribbean probands and their families we developed a structured approach to genetic test result dissemination and family counseling.

Methods

After approval by the University of Miami IRB and the Ethics Committee of the Ministry of Health of Trinidad & Tobago, we prospectively evaluated the active approach to genetic counseling in 32 BC mutation carriers in Trinidad & Tobago in 2015. The intervention consisted of: 1) initial appointment with referring oncologist for results discussion; 2) review of family tree with proband to identify all relatives at 25% or 50% risk (ARR) who should attend the genetic counseling session; 3) preschedule a counseling appointment for ARR within 2 weeks; 4) written invitation to genetic counseling session; 5) handouts of genetic information given to proband to distribute to family members; 6) offer assistance with contacting family members; 7) free genetic testing to ARR who attended the family counseling session.

Results

Twenty-five carriers (78%) consented to enroll in the study. At initial counseling, probands identified 158 ARR, however full family pedigree review at post-result counseling/consultation identified 225 ARR. 101 ARR (64%) attended the information sessions and 76 participants (75.2%) consented to be tested for BC gene mutations. Genetic sequencing revealed 35 ARR (46%) were carriers of at least one mutation. The most frequent reasons for ARR not attending the meetings were: living abroad (18%), unable to be contacted (17%), refusing to participate (17%), not showing up (17%), probands being estranged from ARR (12%) or being afraid to know the results (7%).

Conclusions

In Trinidad & Tobago, a structured approach to the dissemination of genetic test results leads to a significant improvement in the rate of family participation compared to previous efforts (64% in Trinidad & Tobago vs. 9% in the Bahamas).
Title: Treatment decision making, and strategies for coping with financial stress in Indian women diagnosed with breast cancer and their families

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Body: Introduction: In spite of rapid urbanization and modernization the family remains central in the socio-cultural structure of India. The individuals are enmeshed into this unit and tend to be interlinked financially, emotionally and socially. The head of this family unit tends to be a male more often than not. As is well known, despite recent attempts by the governments at the state and centre at providing health coverage for cancer through regional cancer centres, a majority have to raise the money for cancer care by themselves. We have examined the role of the family in treatment decision making and in the strategies employed to raise the money and cope with the financial stress imposed by a diagnosis of breast cancer.

Method: 378 women with breast cancer were enrolled into a longitudinal study at first diagnosis between the years 2008-2012, at two tertiary care hospitals in Bangalore, India. The median follow up as of May 31st 2017 is 78 months with only 2% loss to follow-up over the past 8 years. Follow-up was maintained by frequent meetings between a counselling psychologist (AA) and the patient and/or a family member. The frequency of meetings was monthly during the initial treatment and then quarterly over the next 5 years. Information on demographics was collected during the treatment phase and information on the psychosocial aspects was collected in non-structured interactions subsequently. This information included details of support structure, decision making, and financial arrangements.

Results: This is a predominantly urban cohort with 80% being urban. The median age of patients at first diagnosis was 55 years. Almost all of our patients (99%) had the support of one or more family members. We analysed the pattern of decision making for treatment and in half of all cases either the husband or the son were the decision makers. In an additional 15% daughters and other relatives were the primary decision makers. Approximately a third of women made the decision concerning treatment themselves, and these women tended to be college educated (51% vs 16%) and employed (53% vs 12%). 30% of the patients met the costs incurred through medical insurance plans purchased by the family. Another quarter of patients were able to meet the costs from their savings. 45% had difficulty in finding the money for treatment and 15% took personal loans while 30% had to sell land/gold ornaments or take loans against assets of these sorts. Only (3%) discontinued the treatment due to financial difficulties. As in the case of decision making those who had the financial resources tended to be more educated (41% vs 11%), and were employed (31% vs 21%).

Conclusion: The data from a predominantly urban cohort of breast cancer enrolled between 2008-2012, supports the general belief that in India the family remains the fulcrum of an individual during crises, and not surprisingly education and employment lead to both psychological and economic emancipation of women.
Title: Analyses of racial disparities in genetic testing and surgical management of patients with triple-negative breast cancer in the era of multigene panel testing

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Body: Background
Under-utilization of genetic counseling and testing among African-American (AA) women with breast cancer (BC) has been reported in previous studies, and there are concerns that disparities may widen with further genomic advances. Our objective was to compare AA and Caucasian (CC) patients with triple-negative breast cancer (TNBC) with regards to referral for genetic counseling, patterns of genetic testing, and patient-compliance with genetic counseling recommendations. We chose TNBC since a majority of these patients would qualify for genetic counseling ± testing. We also analyzed for differences between the two races in prevalence of deleterious BC-associated mutations, stage of BC on presentation, and surgical choices.

Methods
In this retrospective medical records-based observational study, we included all patients in our tumor registry with TNBC diagnosed between 09/01/2013 and 02/28/2017. Race, clinical characteristics and details pertaining to genetic counseling and testing were recorded. X² test was used to compare categorical variables. A p-value < 0.05 was considered significant.

Results
477 patients - 358 CC and 96 AA- with TNBC were included. Mean age was 60.3 years. 331 patients met National Comprehensive Cancer Network (NCCN) criteria for genetic counseling - of these, 85.5% had genetic consult order placed, 72.8% attended counseling, and 71.9% underwent genetic testing (multigene panel testing 55.0%, BRCA 1/2 testing 39.9%, single-site testing 2.5%, and multisite-3 testing 2.5%). Between CC and AA, no significant differences were found in the proportion of qualifying patients who had referral for genetic counseling (84.7 vs 87.7%, p=0.562), attended counseling (72.2% vs 73.7%, p=0.816), or underwent genetic testing (72.1% vs 70.1%, p=0.764). The choices of type of genetic tests were also not significantly different between the two groups (p=0.349). Though the prevalence of highly penetrant mutations in breast cancer-associated genes trended to be higher among CC than AA (14.1% vs 9.5%), this difference did not reach statistical significance (p=0.429). In our population, stage of TNBC at presentation was comparable between the two races – 80.4% of CC presented with stage I or II disease compared to 80.2% of AA (p=0.931). The two groups were also comparable with regards to the choices of breast surgery and reconstruction, as shown in the table.

<table>
<thead>
<tr>
<th>Surgery Type</th>
<th>Caucasians</th>
<th>African-Americans</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partial Mastectomy</td>
<td>154(47.4%)</td>
<td>38(44.7%)</td>
<td>0.317</td>
</tr>
<tr>
<td>Unilateral Mastectomy</td>
<td>101(31.1%)</td>
<td>35(41.2%)</td>
<td></td>
</tr>
<tr>
<td>Bilateral Mastectomy</td>
<td>70(21.5%)</td>
<td>12(14.1%)</td>
<td></td>
</tr>
<tr>
<td>Reconstruction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>96(56.1%)</td>
<td>24(51.1%)</td>
<td>0.535</td>
</tr>
<tr>
<td>No</td>
<td>75(43.9%)</td>
<td>23(48.9%)</td>
<td></td>
</tr>
<tr>
<td>Reconstruction Type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Implant</td>
<td>87(90.6%)</td>
<td>19(79.2%)</td>
<td>0.118</td>
</tr>
<tr>
<td>Tissue Flap</td>
<td>9(9.4%)</td>
<td>5(20.8%)</td>
<td></td>
</tr>
</tbody>
</table>

Conclusions
Contrary to previous reports, in this cohort of TNBC patients, we did not identify significant disparities between AA and CC in patterns of referral for genetic counseling, in patient compliance with testing or in type of testing performed. Also, no significant
differences were found between the two races in choices of breast surgery or reconstruction. As a caveat, with an overall insured rate of ~97% across our network, the uninsured population may have been under-represented by our cohort.
Title: Pilot data from the development of the Senior Women's Breast Cancer Clinic at Sunnybrook Odette Cancer Centre

Ines B Menjak¹, Maureen E Trudeau², Rajin Mehta², Fiona McCullock², Bonnie Bristow², Frances Wright², Katie Rice², Leslie Gibson², Mark Pasetka² and Ewa F Szumacher². ¹University of Toronto, Sunnybrook Odette Cancer Centre, Toronto, ON, Canada and ²Sunnybrook Odette Cancer Centre, Toronto, ON, Canada.

Body: Introduction: Women over 70 are a growing demographic of breast cancer patients with specific needs requiring individualized care plans. We developed the interdisciplinary Senior Women's Breast Cancer Clinic (SWBCC) to improve access to a comprehensive geriatric assessment (CGA) and allied health services such as social work, occupational therapy, and pharmacy assistance. After initiation of the SWBCC, we conducted a pilot study using the VES-13 (vulnerable elders survey-13) tool to screen all patients over 70 with the goal of focusing referrals for patients who may benefit most from a CGA. The VES-13 was developed for community-dwelling elders and is validated in oncology patients. The objective of this study is to examine the outcomes of VES-13 screening, determine the medical issues identified by the CGA, and describe the development of this clinic.

Methods: A retrospective review of the clinic from May 2015 - May 2017 was performed using the electronic medical records and paper screening forms. We separately describe the impact of the VES-13 to manage CGA referrals. A score of 3 or greater is a positive screen, and indicates the patient is at risk for death or decline. Non-parametric descriptive statistics were used for statistical analyses.

Results: A total of 25 patients have been seen in the SWBCC for CGA to date. Median age was 83, (range 67-97). A median of two (range 1-4) new medical issues were identified from the CGA for each patient. The most common new diagnoses or issues identified were cognitive impairment (15/25), falls (6/25), neuropathy (4/25), and pain (4/25). The geriatric day program and falls prevention program were common referrals. After the introduction of VES-13 screening, a total of 54 patients were screened. Median age in that group was 78.5 years (range 70-95). The median VES-13 score was 1 (range 0-10). Of the 21 patients screened positive on VES-13, 7 went on to have a CGA. Of the remaining screen-positive patients, 3/21 patients declined SWBCC referral, and the others were not referred at the discretion of the physician. None of the patients with negative VES-13 were referred for CGA. The SWBCC structure was developed to utilize breast cancer-specific resources, whereby geriatricians provide consultation within the oncology space, and the allied health providers were affiliated with the breast centre. Oncology and geriatric administrative staff organized bookkeeping to better coordinate schedules between the two disciplines. The geriatricians supervised trainees for the CGA, and follow-ups took place at SWBCC or in the geriatric outpatient clinic. Clinic coordinators affixed the VES-13 tool to all new patient charts for those aged ≥70. Nursing resources were dedicated to assist patients with VES-13 if needed, and document scores in the electronic medical record.

Conclusions: A dedicated clinic for seniors with breast cancer providing geriatric assessment can identify important undiagnosed medical issues that warrant intervention or monitoring during breast cancer treatment. The VES-13 screening tool provides useful information to help manage resources for geriatrics referral. A prospective trial examining the role of CGA in decision-making for adjuvant chemotherapy is underway in this clinic.
Title: Impact of delay in breast cancer diagnosis and treatment according to health insurance status in southwest Brazil and Houston, Texas

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Body: Background: Access to medical care vary across the world and is related to different health systems with an impact in recurrence. Objective: To evaluate disparities in breast cancer (BC) diagnosis and treatment between public and private services in southwest Brazil and at two public safety net hospitals in Houston, Texas. Methods: Women diagnosed with BC stages I-III between 2009 to 2011, and treated at the four hospitals in Brazil and two health centers in US were included. All statistical analyses were performed in R studio software, and p<0.05 was considered significant. Results: 1245 women were included: 967 from public health system (PHS) (20.3% from Houston, Texas) and 274 from private system (PS). Recurrence rate was higher in PHS (14.6% vs. 2.6%, p<0.001)

Table 1. Clinical and demographic characteristics of the patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Public (%), n=967</th>
<th>Private (%), n=274</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discovery of BC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>By patient</td>
<td>530 (54.8)</td>
<td>92 (33.5)</td>
<td></td>
</tr>
<tr>
<td>Routine exam</td>
<td>87 (9)</td>
<td>109 (39.8)</td>
<td></td>
</tr>
<tr>
<td>Screening mammography</td>
<td>270 (27.9)</td>
<td>23 (8.4)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>80 (8.3)</td>
<td>50 (18.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Initial treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>687 (71)</td>
<td>241 (88)</td>
<td></td>
</tr>
<tr>
<td>Neo-adjuvant chemotherapy</td>
<td>224 (23.2)</td>
<td>27 (9.8)</td>
<td></td>
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<tr>
<td>Neo-adjuvant hormone therapy</td>
<td>23 (2.3)</td>
<td>27 (9.8)</td>
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</tr>
<tr>
<td>Not available</td>
<td>33 (3.4)</td>
<td>3 (1.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Clinical Stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>293 (30.3)</td>
<td>113 (41.2)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>342 (35.4)</td>
<td>52 (19)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>271 (28)</td>
<td>15 (5.5)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>61 (6.3)</td>
<td>94 (34.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Subtype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR+/HER2 -</td>
<td>561 (58)</td>
<td>192 (70.1)</td>
<td></td>
</tr>
<tr>
<td>HR-/HER2+</td>
<td>108 (11.1)</td>
<td>29 (10.6)</td>
<td></td>
</tr>
<tr>
<td>HR-/HER2+</td>
<td>76 (7.9)</td>
<td>14 (5.1)</td>
<td></td>
</tr>
<tr>
<td>Triple negative</td>
<td>149 (15.4)</td>
<td>28 (10.2)</td>
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<tr>
<td>Unknown</td>
<td>73 (7.6)</td>
<td>11 (4)</td>
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<tr>
<td>Symptomatic at Diagnosis</td>
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</tr>
<tr>
<td>Yes</td>
<td>591 (61.1)</td>
<td>100 (36.5)</td>
<td></td>
</tr>
</tbody>
</table>
Considering the interval in weeks: symptoms to diagnosis, diagnosis to first treatment (either surgery or neoadjuvant chemotherapy), diagnosis to first systemic treatment, diagnosis to surgical treatment and diagnosis to radiotherapy were longer in public patients (24.1 vs. 8.7; 11.1 vs. 3.5; 18.6 vs. 9.8; 16.9 vs. 5.6; 51.4 vs. 26.1; \( p < 0.001 \)).

Table 2. Delay disparities between public and private health system

<table>
<thead>
<tr>
<th></th>
<th>Public</th>
<th>Private</th>
<th>( p )</th>
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</thead>
<tbody>
<tr>
<td>Symptoms to diagnosis</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients</td>
<td>575</td>
<td>146</td>
<td></td>
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<tr>
<td>Time (weeks)</td>
<td>24.1 (0.4-104.9)</td>
<td>8.7 (0.0-43.7)</td>
<td>&lt;0.001</td>
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<tr>
<td>Diagnosis to first treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients</td>
<td>663</td>
<td>180</td>
<td></td>
</tr>
<tr>
<td>Time (weeks)</td>
<td>11.1 (2.0-31.5)</td>
<td>3.5 (0.0-11.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diagnosis to first systemic treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients</td>
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<td>106</td>
<td></td>
</tr>
<tr>
<td>Time (weeks)</td>
<td>18.6 (2.6-44.7)</td>
<td>9.8 (1.9-29.3)</td>
<td>&lt;0.001</td>
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<tr>
<td>Diagnosis to surgical treatment</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Number of patients</td>
<td>657</td>
<td>178</td>
<td></td>
</tr>
<tr>
<td>Time (weeks)</td>
<td>16.9 (3.4-45.6)</td>
<td>5.6 (0.0-32.9)</td>
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<tr>
<td>Diagnosis to radiotherapy</td>
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<td></td>
</tr>
<tr>
<td>Number of patients</td>
<td>465</td>
<td>127</td>
<td></td>
</tr>
<tr>
<td>Time (weeks)</td>
<td>51.4 (18.7-88.4)</td>
<td>26.1 (5.6-66.4)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

In multivariate analysis, PHS (HR 1.72; 95% CI 1.34-1.88; \( p \) adj=0.003), presence of symptoms (HR 2.29; 95% CI 1.39-3.78; \( p \) adj=0.001), clinical stage III (HR 1.62; 95% CI 1.35-1.93; \( p \) adj<0.001), and triple negativity and HER2neu positivity (1.18; 95% CI 1.03-1.35; \( p \) adj=0.021) were all associated with a higher recurrence rate.**Conclusions:** There were significant disparities between PHS and PS. Women in the PHS presented higher rates of recurrence, advanced clinical stages at diagnosis, symptoms and more aggressive subtypes by IHC. additionally, the interval between symptoms to diagnosis and diagnosis to treatments was longer in PHS.
Body: Background: Delays in diagnosis and treatment is associated with recurrence. Access to medical care in public health systems (PHS) may vary across Southwest Brazil and Houston, Texas. Objective: To evaluate disparities in breast cancer (BC) diagnosis and treatment between public services in southwest Brazil and at a public safety net hospital in Houston, Texas. Methods: Women diagnosed with BC stages I-III between 2009 to 2011, and treated at the four hospitals in Brazil and two health centers in US were included. All statistical analyses was performed in R studio, and $p<0.05$ was considered significant. Results: 967 women from PHS were included: 778 from Brazil and 189 from US. Recurrence rates were not significantly different (15.9% vs. 9.5%, $p=0.233$)

Table 1. Clinical and demographic characteristics of the patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Brazil (%)</th>
<th>US (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Brazil (n=778)</td>
<td>US (n=189)</td>
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<tr>
<td>Discovery of BC</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>By patient</td>
<td>443 (56.9)</td>
<td>87 (46)</td>
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<tr>
<td>Routine exam</td>
<td>82 (10.5)</td>
<td>5 (2.7)</td>
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</tr>
<tr>
<td>Screening mammography</td>
<td>207 (26.6)</td>
<td>63 (33.4)</td>
<td>&lt;0.001</td>
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<tr>
<td>Other</td>
<td>46 (6)</td>
<td>34 (17.9)</td>
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<tr>
<td>Initial treatment</td>
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<tr>
<td>Surgery</td>
<td>587 (75.4)</td>
<td>100 (52.9)</td>
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<td>Neo-adjuvant chemotherapy</td>
<td>176 (22.6)</td>
<td>48 (25.4)</td>
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<td>Neo-adjuvant hormone therapy</td>
<td>9 (1.1)</td>
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<td>Unknown</td>
<td>6 (0.9)</td>
<td>27 (14.3)</td>
<td>&lt;0.001</td>
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<tr>
<td>Clinical Stage</td>
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</tr>
<tr>
<td>I</td>
<td>234 (30.1)</td>
<td>59 (31.2)</td>
<td></td>
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<tr>
<td>II</td>
<td>289 (36.8)</td>
<td>56 (29.7)</td>
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<tr>
<td>III</td>
<td>239 (30.7)</td>
<td>32 (16.9)</td>
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<td>HR+/HER2 -</td>
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<td>HR+/HER2+</td>
<td>91 (11.7)</td>
<td>17 (9)</td>
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<td>HR-/HER2+</td>
<td>66 (8.5)</td>
<td>10 (5.3)</td>
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<tr>
<td>Triple negative</td>
<td>123 (15.8)</td>
<td>26 (13.8)</td>
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<tr>
<td>Unknown</td>
<td>31 (4)</td>
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<td>Symptoms</td>
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<tr>
<td>Yes</td>
<td>492 (63.2)</td>
<td>99 (52.4)</td>
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</tr>
<tr>
<td>No</td>
<td>248 (31.9)</td>
<td>58 (30.7)</td>
<td></td>
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</table>
Table 2. Delay disparities in public health system between Brazil and US

<table>
<thead>
<tr>
<th></th>
<th>Brazil</th>
<th>US</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptoms to diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients</td>
<td>473</td>
<td>102</td>
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<tr>
<td>Time (weeks)</td>
<td>24.4 (0.5-102.6)</td>
<td>22.8 (0.1-124.8)</td>
<td>&lt;0.001</td>
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<tr>
<td>Diagnosis to first treatment</td>
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<td></td>
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<tr>
<td>Number of patients</td>
<td>546</td>
<td>117</td>
<td></td>
</tr>
<tr>
<td>Time (weeks)</td>
<td>10.8 (1.9-31.7)</td>
<td>12.2 (3.1-26.4)</td>
<td>&lt;0.001</td>
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<td>Diagnosis to first systemic treatment</td>
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<tr>
<td>Number of patients</td>
<td>459</td>
<td>67</td>
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</tr>
<tr>
<td>Time (weeks)</td>
<td>18.9 (2.4-45.6)</td>
<td>16.1 (3.3-38.6)</td>
<td>&lt;0.001</td>
</tr>
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<td>Diagnosis to surgical treatment</td>
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<td>Number of patients</td>
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<td>116</td>
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<tr>
<td>Time (weeks)</td>
<td>16.4 (3.1-43.1)</td>
<td>19.2 (4.5-51.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diagnosis to radiotherapy</td>
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<td></td>
<td></td>
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<tr>
<td>Number of patients</td>
<td>413</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>Time (weeks)</td>
<td>51.8 (19.4-89.7)</td>
<td>47.8 (14.9-84.8)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

However, considering the interval in weeks: diagnosis to first treatment (either surgery or neoadjuvant chemotherapy) and diagnosis to surgical treatment were shorter in Brazil PHS patients (10.8 vs. 12.2 and 16.4 vs. 19.2, p<0.001). When considering surgery as initial treatment, treatment in Brazil PHS and stage III disease at diagnosis were associated with worse prognosis (HR: 1.91, 95%CI: 1.38 – 1.99, padj=0.015; HR 1.44, 95%CI:1.11-1.87, padj=0.006). **Conclusions:** There were significant disparities between Brazil and US PHS. Women in Brazil PHS presented higher rates of advanced clinical stages at diagnosis and diagnosis by routine exam, while US PHS present higher rates of diagnosis by screening mammography. When considering surgery as initial treatment, Houston PHS women had a better prognosis.
Title: Survival outcomes related to health care coverage in metastatic breast cancer in Brazil: A sub-analysis from the LACOG-0312 study

Gustavo Werutsky¹, Facundo Zaffaroni¹, Deise Uema², Eduardo Cronenberger³, Vladimir C Cordeiro de Lima⁴, Rosane O de Sant'ana⁵, José Bines⁶, Patricia X Santi⁷, Renata S Góes⁸, Pedro Liedke⁹, Maria LM Batista¹⁰, Vanessa Dybal¹¹, Yeni V Nerón¹², Carlos A Beato¹³, Giuliano Borges¹⁴, Juliana Giacomazzi¹⁵, Lucas V dos Santos¹⁶, Gustavo Ismael¹⁷, Daniela D Rosa¹⁸, Alan Azambuja¹⁹, Diocésio Andrade²⁰, Jeovany Martinez-Mesa²¹, Marcio Debiassi¹ and Carlos H Barrios¹. ¹Latin American Cooperative Oncology Group (LACOG), Porto Alegre, Brazil; ²Instituto do Câncer do Estado de São Paulo (ICESP), São Paulo, Brazil; ³Centro Regional Integrado de Oncologia (CRIO), Fortaleza, Brazil; ⁴A.C. Camargo Cancer Center, São Paulo, Brazil; ⁵Hospital do Câncer do Ceará - ICC, Fortaleza, Brazil; ⁶Instituto Nacional de Câncer (INCA), Rio de Janeiro, Brazil; ⁷Centro de Estudos e Pesquisa de Hematologia (CEPHO), Santo André, Brazil; ⁸Instituto Brasileiro de Controle do Câncer (IBCC), São Paulo, Brazil; ⁹Hospital de Clínicas de Porto Alegre (HC PA), Porto Alegre, Brazil; ¹⁰Núcleo de Oncologia da Bahia (NOB), Salvador, Brazil; ¹¹Clínica AMO, Salvador, Brazil; ¹²Centro de Pesquisa Clínica – CEPON, Florianópolis, Brazil; ¹³Hospital Amaral Carvalho, Jaú, Brazil; ¹⁴Centro de Novos Tratamentos Itajaí - Clínica de Neoplasias Litoral, Itajaí, Brazil; ¹⁵Hospital Tacchini, Bento Gonçalves, Brazil; ¹⁶Instituto de Ensino e Pesquisa Síao Lucas, São Paulo, Brazil; ¹⁷Centro de Hematologia e Oncologia de Rio Claro, Rio Claro, Brazil; ¹⁸Hospital Moinhos de Vento, Porto Alegre, Brazil; ¹⁹Hospital do Câncer Mãe de Deus, Porto Alegre, Brazil; ²⁰Instituto Oncológico de Ribeirão Preto (InORP), Ribeirão Preto, Brazil and ²¹MED, Escola de Medicina, Passo Fundo, Brazil.

Body: Background
Metastatic breast cancer (MBC) is an incurable disease in which latest therapies have evolving and improving patients survival. Inequities in the access to optimal treatment and shorter survival of BC by type of health care coverage were previously reported in an observational study in Brazil. In Brazil patients with private health coverage have access to the most recent therapies, however the public health system does not provide several therapies approved for the treatment of MBC such as everolimus, trastuzumab, eribulin, TDM-1, pertuzumab among others. The present analysis aims to analyze the impact of the type of health care coverage on survival outcomes of patients with MBC.

Methods
LACOG-0312 is a retrospective cohort study that enrolled patients with metastatic or locally advanced/recurrent unresectable BC diagnosed during 2012 in Brazil. Overall survival was defined as the time from the diagnosis of MBC and death from any cause. Comparisons were made using the Kaplan-Meier method based on the type of health care coverage (public vs. private). Cox regression analysis was performed for identification of independent prognostic factors associated with overall survival.

Results
A total of 634 patients with MBC were included in the study. Baseline characteristics by type of health care coverage was similar for visceral disease (43% in public and 44% in private, p=0.78), age at MBC diagnosis (median 62 years in public and 64 years in private, p=0.25), BC subtype (p=0.89), however more patients public insured were metastatic at diagnosis (42% vs. 33%) and had performance status >= 2 (12% vs. 3%). The proportion of patients that received any first-line systemic therapy was similar in both groups (95.2% in public and 95.5% in private, p=1.0), however more patients with private insurance received second (82% vs. 71.6%, p=0.013) and third line (56% vs. 45%, p=0.024) therapy compared to public health covered patients. OS from the date of MBC diagnosis in whole population was 36 months. There was no difference in terms of OS between private (42 months) and public (35 months) health insured patients (p=0.65). OS by BC subtype was 15 months for triple negative, 23 months in HER2 positive, 44 and 42 months for Luminal A and B respectively. There was no difference in OS by type of health insurance coverage in any BC subtypes. In a multivariate analysis type of health care coverage did not associate with survival, only triple negative (HR (95% CI) – 3.495 (2.448 - 4.989); p <0.001), HER2 positive (HR (95% CI) - 2.287 (1.394 - 3.572); p = 0.001) BC subtypes and visceral metastases (HR (95% CI) – 1.413 (1.075 - 1.858); p <0.013) were correlated with a worse survival.

Conclusion
Our study suggests that health care coverage is not associated with survival outcomes in patients with MBC. Potential differences in the access to optimal systemic treatments may not play a significant role in the survival of these patients.
Real-world studies addressing the impact of new cancer therapies for different BC subtypes in MBC are needed.
Title: Medismart-Oncosmart: A low-cost private initiative to improve health care access in the middle-income country of Costa Rica. Overview of the breast cancer screening program

Andres Wiernik¹, Leonardo Lami Casaus¹, Marissa Durman¹,² and Roberto Herrera Guido¹. ¹Hospital Metropolitano, San Jose, Costa Rica and ²Hennepin County Medical Center, Minneapolis, MN.

Body: Background: Costa Rica is a middle-income country in Central America with a public, government run, universal health care system which provides coverage to over 95% of the population. Despite being recognized as one of the best health care systems in Latin America, recent national reports suggest that up to 15% of the population is currently on a waiting list for a medical visit or procedure. The impact of the national breast cancer screening program is unknown as the country's health care system and the national cancer registry fail to report statistical variables like screening waiting lists, breast screening coverage, stage at diagnosis, time from screening to biopsy, breast cancer subtypes, breast cancer mortality by stage, among others.

Methods: In 2014, the private health plan Medismart partnered with the private Hospital Metropolitano network with the mission of developing a low-cost health care system that provides efficient access and affordable health care services to its affiliates. Medismart's health care plan costs $12 a month per member ($6 per additional affiliated family member) and uses a copay model for health care services utilized by its beneficiaries. In 2016, Oncosmart, a program of Medismart, was developed with the goal of implementing population based screening, diagnostic procedures, cancer treatment, and survivorship care to its members. The program has no restrictions on pre-existing conditions.

Results: From January 2015 to April 2017, Medismart subscribed 50,122 members. During the first 12 months of launching the breast screening program, 3634 women (32% of target population of 11058 women over 40 years old) underwent breast cancer screening mammography, breast ultrasound or both at the Hospital Metropolitano network. The copayment price of mammography screening for Medismart members is $26 and the copayment price for breast ultrasound screening is $38. Of the women who underwent screening in this period, 153 required a breast biopsy and all biopsies performed at the Metropolitano hospital were completed within 30 days from the abnormal imaging study.

Conclusion: Medismart-Oncosmart is a low cost and efficient private initiative that improves access to breast cancer screening in Costa Rica.
Title: Disparities in use of adjuvant radiotherapy following lumpectomy among California regions

Ryan J Huang¹, Rita A Mukhtar¹ and Michael Alvarado¹. ¹University of California, San Francisco, San Francisco, CA.

Body: Background
Women undergoing lumpectomy for invasive breast cancer typically receive radiotherapy (RT) to reduce risk of recurrence. Previous studies have reported disparities in the utilization of RT by race, socioeconomic status (SES), and age. In this study, we evaluate whether various sociodemographic factors are associated with use of RT in the different regions of California.

Methods
Utilizing data from the California Cancer Registry, the authors identified cases of women whose first primary invasive breast cancer was diagnosed between January 1, 2007 and December 31, 2012, whose primary surgery was breast conserving surgery, and for whom information was complete (n = 71,767). Multivariate logistic regression was used to determine whether demographic factors (SES, race, payer status, age) were significantly associated with use of RT following lumpectomy (p < .05), adjusting for tumor characteristics (size, stage, grade, hormone receptor status, and nodal status).

Results
In three out of eight regions in California, black race was associated with decreased odds of RT use (San Francisco, OR = 0.79, 95% CI = 0.68-0.92; Desert Sierra, OR = 0.72, 95% CI = 0.58-0.90; Los Angeles, OR = 0.78, 95% CI = 0.70-0.87). In Sacramento and Los Angeles regions, lower socioeconomic status was associated with declined odds of RT use. Age (70 years or older) was also associated with lower likelihood of RT use across all regions.

Conclusions
Even after accounting for payer status, racial and socioeconomic disparities persist in the use of RT. These disparities, previously documented in the time period of 2000-2007, have not disappeared. Hispanic race was not shown to be associated with decreased odds of RT use in Los Angeles, contrary to the results of a previous study. Women aged 70 years or older are less likely to receive RT.
Body: BACKGROUND

Breast cancer is the most common cancer among Brazilian women. HER-2 targeted therapy improves overall survival in HER-2 overexpressing patients but immunohistochemistry testing for HER2 is not standardized in Brazil and is not available universally. In Brazil the health system includes a public and private sector. The aim of our study was to delineate the patterns of testing of HER-2 over time in Brazil in both of these settings and to determine if any disparities exist in testing and treatments received.

METHODS

Observational, retrospective study involving practice patterns of over 2000 cancer physicians in Brazil. We obtained de-identified data from a commercial database, which included 54,829 patients with breast cancer treated between 2012 and 2016. We analyzed the frequency of HER-2 testing, the percentage of positive results and the most common treatments used in the first line setting in both the private and public sector. The chi-squared test was used for proportions.

RESULTS

HER-2 testing was frequently performed in both the private and public sector (87% vs. 81%, p<0.0001. Between 2012 and 2016 most patients had HER-2 testing (88%, 73%, 79%, 90% and 88%, respectively) but coverage was not universal. The percentage of HER-2 positivity was 25%. The most common first line regimens used were docetaxel/trastuzumab, paclitaxel/trastuzumab and trastuzumab monotherapy. In the private sector trastuzumab/pertuzumab/docetaxel was the most commonly used regimen. In the public sector taxanes were frequently used as monotherapy without HER-2 directed therapy.

CONCLUSIONS

To our knowledge this is the largest dataset assessing HER-2 testing and treatment patterns in Brazil. The frequency of testing has remained stable over the last 5 years, but is higher in the private sector and this finding was highly statistically significant. There are also differences in the regimens used in the private vs. public sector. Pertuzumab was approved in 2013 in the US and its use has increased in Brazil over the last two years. This trend however was only seen in the private sector. In the public sector there is still significant use of chemotherapy without HER-2 directed therapy despite HER-2 overexpression, which is possibly related to the restricted access of anti-HER2 therapy in the public health system for metastatic patients. Taxanes are used widely in both the public and private sector, which is possibly related to the availability of generics.

<table>
<thead>
<tr>
<th>Year</th>
<th>Private</th>
<th>Public</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tested</td>
<td>%</td>
<td>Non Tested</td>
</tr>
<tr>
<td>2012</td>
<td>4,285</td>
<td>92%</td>
</tr>
<tr>
<td>2013</td>
<td>3,711</td>
<td>76%</td>
</tr>
<tr>
<td>2014</td>
<td>4,592</td>
<td>80%</td>
</tr>
<tr>
<td>2015</td>
<td>4,688</td>
<td>94%</td>
</tr>
<tr>
<td>2016</td>
<td>4,126</td>
<td>91%</td>
</tr>
<tr>
<td>------------------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>Tested</td>
<td>5,391</td>
<td>3,996</td>
</tr>
<tr>
<td>%</td>
<td>85%</td>
<td>71%</td>
</tr>
<tr>
<td>Non Tested</td>
<td>949</td>
<td>1,661</td>
</tr>
<tr>
<td>%</td>
<td>15%</td>
<td>29%</td>
</tr>
<tr>
<td>Total</td>
<td>6,340</td>
<td>5,657</td>
</tr>
</tbody>
</table>
Title: Disparities in the risk of mortality in breast cancer based on health insurance status

Stuthi Perimbeti, Hareesha Chakunta, Longjian Liu, Kristine Ward, Maneesh Jain and Michael Styler. 1Drexel University College of Medicine, Philadelphia, PA and 2Drexel University School of Public Health, Philadelphia, PA.

Body: In 2007 Foley et al showed that low-income Canadian residents had a survival advantage over low-income US residents, which was attributed to the equal access to medical care in Canada's universal health care system. Niu et al. found that uninsured and Medicaid insured patients with breast, cervical, colorectal, head and neck, lung, prostate or uterine cancer have higher mortality rates compared to patients with private insurance or Medicare. As substantial proportions of the US population are uninsured or enrolled in Medicaid, we examined the association between in-hospital mortality and different primary payers in patients with breast cancer.

Methods
Adult female admissions (adm) with a primary diagnosis of breast cancer between 1999 and 2014 were extracted from the National Inpatient Sample database using the ICD-9 code 174.9 (N=98631, for a weighted N=484859). The sample was weighted to approximate the full inpatient population of the US over the time period. To minimize the effect of changes in mortality rates based on insurance status over the time interval studied, we grouped the adm into three categories: Group 1 for adm from 1999 to 2003, Group 2 2004 to 2008, and Group 3 2009 to 2014. Chi-Square analysis was done to determine the in-hospital mortality rates by payer group and Cox Proportional Hazard regression was used to determine the hazard of death (HR) within 30 days of adm by payer.

Results

<table>
<thead>
<tr>
<th>Year Group</th>
<th>Medicare</th>
<th>Medicaid</th>
<th>Private Insurance</th>
<th>Selfpay/Uninsured</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.83</td>
<td>6.77</td>
<td>3.43</td>
<td>10.14</td>
<td>0.0001</td>
</tr>
<tr>
<td>2</td>
<td>4.58</td>
<td>7.55</td>
<td>4.95</td>
<td>9</td>
<td>0.0001</td>
</tr>
<tr>
<td>3</td>
<td>2.84</td>
<td>3.46</td>
<td>3.02</td>
<td>10.26</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Year Group</th>
<th>Medicaid vs Medicare</th>
<th>P value</th>
<th>Uninsured vs Medicare</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.03 (1.49, 2.76)</td>
<td>0.0001</td>
<td>3.2 (2.49, 4.22)</td>
<td>0.0001</td>
</tr>
<tr>
<td>2</td>
<td>1.56 (1.19, 2.46)</td>
<td>0.0001</td>
<td>2.9 (2.20, 3.80)</td>
<td>0.0001</td>
</tr>
<tr>
<td>3</td>
<td>2.55 (1.99, 3.27)</td>
<td>0.0001</td>
<td>7.76 (1.99, 3.27)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

The number of adm with Medicare or Private insurance were higher than those with Medicaid or Selfpay/uninsured. The in-hospital mortality was highest for Selfpay/uninsured, followed by admissions with Medicaid. After controlling for age, race, median income and comorbidities, the HR was significantly higher in the Medicaid and selfpay/uninsured admissions compared to Medicare admissions. In Group 1, compared to Medicare adm the HR was 103% higher for Medicaid and 220% higher for
uninsured. In Group 2, the HR was 56% higher for Medicaid and 196% higher for uninsured. In Group 3, it was 155% higher for Medicaid and 676% higher in uninsured.

**Conclusion**

Even after controlling for other factors which are implicated in the mortality, the HR is significantly higher in Medicaid and uninsured admissions when compared with Medicare enrolled admissions with breast cancer. Equitable distribution of health was one of the “Aims for Improvement” in the Institute of Medicine’s 2001 report, but our results suggests that this has not yet been achieved. Insurance status still appears to play a crucial role in patient outcomes and should be considered as a metric of equitable care. More scientific research is needed in the area of differential receipt of standard therapy in cancer patients considering the limitations of our study.
Title: Quality indicators in breast cancer care: Experience of a breast cancer multidisciplinary unit at Instituto Nacional de Ciencias Médicas y Nutrición. Dr Salvador Zubirán, (INCMNSZ) Mexico City

Alejandra Amengol-Alonso1, Yanin Chávarri-Guerra1, Ruben Córtes-González1, Heriberto Medina-Franco1, A Espinoza de los Monteros1, Armando Gamboa-Domínguez1, Fernando Candanedo-González1, Christian Flores-Balcazar1, Rubi Ramos1, Rosaura Fuentes-Corona1, Monica Chapa-Ibarguengoitia1, Paola Balboa-González1, Alexa Bazúa-Gerez2 and Eucario León-Rodríguez1.

1Instituto Nacional de Ciencias Médicas y Nutrición "Salvador Zubirán" (INCMNSZ), Mexico City, Tlalpan, Mexico. and 2Facultad de Medicina Universidad Autónoma de San Luis Potosí, San Luis Potosí, Mexico.

Body: Background: In 2012, GLOBOCAN reported that the incidence of BC among Mexican women was 35.4 per 100,000 inhabitants. According to some published studies, the five-year overall survival among Mexican breast cancer patients (BCP) treated at governmental facilities is about 75-80%. As a result of an increased access to oncology treatments, multidisciplinary breast cancer units (MBCU) have been developed all over the country aiming to improve outcomes in BCP, however, to date, information regarding quality of care measured by international criteria is lacking. In 2011 our institution was a pioneer in establishing MBCU. The aim of the present study was to describe quality indicators in breast cancer care at our MBCU.

Patients and methods: This is a prospective study including 357 breast cancer patients from January 2011 to December 2016. 111 variables were recorded for each patient. Based on the 17 items to evaluate quality in BCP of 2008 European Society of Breast Cancer Specialist (EUSOMA), the EUSOMA-based evaluated criteria were: 1) Completeness of clinical and imaging diagnostic work-up, 2) Multidisciplinary discussion, 3) Waiting times, 4) Appropriate surgical approach (breast conservative surgery in IBC ≤ 3 cm), and 5) Post-operative radiotherapy. The analysis was performed using SPSS software v.21.

Results: Three hundred and fifty-five patients were included (99.4% women). The median age was 57 years (P25-75 49-66 years). Stages were classified as: cTNM stage I (32.2%), stage II (34.7%), stage III (10.6%), and stage IV (18.2%). The results of the EUSOMA-based criteria were: 1) 95% of the cohort completed clinical and imaging diagnostic work-up (EUSOMA minimum standard 90%, target 95%, LoE III); 2) a multidisciplinary discussion at the time of diagnosis was established in 97% of BCP (EUSOMA minimum standard 90%, target 99%, LoE IV); 3) the median waiting time from histological diagnosis to primary treatment was 3.8 weeks (median time for surgery and systemic treatment was 4.5 and 1.4 weeks, respectively) (EUSOMA cut point 6 weeks, minimum standard 75%, target 90%, LoE IV); 4) surgical treatment was performed as first-line therapy in 196 BCP (127 (64.6%) conservative surgery and 69 (35.4%) mastectomy) (EUSOMA conservative surgery minimum standard 70%, target 80%, LoE I); 5) 95% of BCP with an indication for adjuvant radiotherapy had a median waiting time of 1.5 weeks (EUSOMA minimum standard 90%, target 95%, LoE I).

Conclusion: According to these results, our MBCU meets the minimum standards of quality in 5 EUSOMA indicators. However, it is important to highlight that our MBCU is small, analyzing 100 new patients/year but comprising with enough infrastructure and staff for this demand; for this reason, real data representing other Mexican or Latin American MBCUs might not be accurate. Furthermore, to increase the quality of breast cancer care, all EUSOMA objectives should be measured (always adapting to the evaluated a breast cancer population) and only in this way areas of opportunity for improvement could be identified. The present study highlights the need to carry out specific research in breast cancer care quality in low and middle income countries.
Disparity of epidemiological and clinicopathological features of breast cancer between urban and rural women in northern China

Jinnan Gao¹, Wanzhi Song¹, Xiaojun Zhang¹ and Fan Guo¹. ¹Shaxi Dayi Hospital, Taiyuan, Shanxi, China.

Body: Introduction: Breast cancer is the most frequently diagnosed cancer in women worldwide, in particular, the incidence of breast cancer is rapidly increasing in China. As the gap in socioeconomic statuses, such as financial resources and accessibility to health care, between residents in urban and rural regions in China is expanding, which inevitably affects life styles that is associated with the pathogenesis of breast cancer, it is imperative to understand potential urban-rural disparity in epidemiological and clinicopathological characteristics and treatment options of breast cancer patients in China.

Patients and Method: A cohort of 660 patients who were diagnosed with invasive breast cancer and received treatment between March 2012 and October 2016 at the department of breast surgery of Shanxi Dayi hospital were recruited to this study. These included 368 and 292 patients from urban and rural districts, respectively. The demographic characteristics, clinicopathological features and therapeutic options were analyzed.

Results: There was no significant difference between the two groups in median age (53 years vs 53.5 years, P = 0.098). The mean age of menarche in the urban group was significant earlier than the rural group (14.6 years vs 15.1 years, P = 0.004). The number of pregnancy and birth in the urban group was less than the rural group (P = 0.015, P=0.025; respectively). No significant difference was found between the two groups in menopausal status, lactation and breast cancer family histories. Compared with urban patients, those from rural regions took longer time to have their confirmed diagnoses made after the first symptoms noticed (90 days vs 60 days, P = 0.078). The tumour sizes were significantly different between the two groups: rural patients commonly presented with tumours with larger bulks than those in the urban group (3.12cm versus 2.25cm, P=0.038). Moreover, more tumours staged at T3 were found in the rural group (15.1 % versus 4.6%, P=0.038). Regional lymph node involvement was also more common in rural patients (50% versus 34.8%, P=0.003). There was no significant difference between the two groups in terms of histological grades, molecular subtypes and vascular infiltration. Patients in the urban group were more commonly treated with breast conserving surgery compared to those in the rural group (47.1% versus 33.3%, P=0.007), whereas rural patients were less likely to receive treatment with targeted therapy compared with those from urban region (40.6% versus 61.9%, P=0.069).

Conclusions: Compared with patients living in urban areas, those from rural regions in northern China more frequently presented with advanced breast cancers. This suggests that more attention should be paid to rural women in northern China in regard to breast cancer prevention, detection, and treatment. The relatively high incidence of breast cancer in urban regions is conceivably related to changes in life styles associated with breast cancer risk. Further studies will identify the difference in treatment response and prognosis among the four subtypes in this cohort.
Body: Introduction:
Anxiety is a common symptom in patients with newly diagnosed cancer. Patients with high levels of anxiety have been shown to choose more invasive surgeries in various cancer settings. Rates of contralateral prophylactic mastectomy in the adjuvant setting remain high, despite offering no survival advantage. In the neoadjuvant setting, patients have more time for decisions regarding final surgery. If anxiety is playing a role in their decisions, this could be addressed while on neoadjuvant therapy (NAT). However, the impact of anxiety at initial diagnosis on surgical decision making in this setting has not yet been studied.

Methods:
Data collected from a prospective institutional database of breast cancer patients treated with NAT at the British Columbia Cancer Agency (BCCA) were utilized to identify all patients. Information was extracted from this database with regards to patient and tumour characteristics, initial surgical plan, and final surgery performed. This was cross referenced with patient self-reported anxiety, which was extracted from the Edmonton Symptom Assessment System (ESAS) and the Psychosocial Screen for Cancer (PSSCAN) forms administered at initial consultation. Patients were assigned a score of 0 to 3 based on their answers to the ESAS anxiety scale and the PSSCAN anxiety questions, and whether or not they had any concerns related to their care. Patients were excluded if they had bilateral breast cancer, BRCA mutation or referral to the Hereditary Cancer Program, did not receive NAT or undergo breast surgery, or did not complete the forms. Fisher's exact tests were applied for statistical analysis.

Results:
From 2012-2016, 361 potential patients were identified for this study. A total of 203 patients met eligibility criteria. 93 patients (46%) had low anxiety (score 0 or 1) and 110 patients (54%) had high anxiety (score 2 or 3). Patients with high self-reported anxiety at initial consultation were significantly more likely to undergo bilateral mastectomy for unilateral disease and mastectomy for breast conserving surgery (BCS) eligible disease than those with low self-reported anxiety at initial consultation (37.3% VS 18.3%; p=0.003). No significant differences in treatment times (time interval between biopsy to chemotherapy, chemotherapy to surgery, and surgery to radiation) or investigations were identified when comparing high and low anxiety patients.

Anxiety level and type of surgery performed

<table>
<thead>
<tr>
<th>Type of Surgery</th>
<th>Anxiety Level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low (n=93)</td>
</tr>
<tr>
<td></td>
<td>High (n=110)</td>
</tr>
<tr>
<td>Bilateral Mastectomy and Mastectomy for BCS eligible disease</td>
<td>17 (18.3%)</td>
</tr>
<tr>
<td></td>
<td>41 (37.3%)</td>
</tr>
<tr>
<td>BCS and Mastectomy for non-BCS eligible disease</td>
<td>76 (81.7%)</td>
</tr>
<tr>
<td></td>
<td>69 (62.7%)</td>
</tr>
</tbody>
</table>

\( p = 0.0031 \)

Conclusion:
High anxiety scores lead to a 19% increase in bilateral mastectomies in patients without bilateral disease and mastectomies in patients eligible for BCS compared to patients with low anxiety (\( p = 0.003 \)). These findings suggest that self-reported anxiety levels can inform and assist physicians to identify patients who are more likely to undergo aggressive surgery and may need further counselling and support services. Future work should examine the effects of counselling intervention in patients with high anxiety on surgical decisions.
Title: Acceptability and usability of iPrevent, a web-based decision support tool for assessment and management of breast cancer risk

Kelly-Anne Phillips\(^1\), Louisa Lo\(^1\), Mathias Bressel\(^1\), Ian M Collins\(^2\), Jon Emery\(^3\), Prue Weideman\(^1\), Louise Keogh\(^3\), Emma Steel\(^3\), Adrian Bickerstaffe\(^3\), G Bruce Mann\(^4\), Alison Trainer\(^1\), John L Hopper\(^3\), Antonis C Antoniou\(^5\), Jack Cuzick\(^6\) and Phyllis Butow\(^7\).

\(^1\)Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia; \(^2\)Deakin University, Geelong, Victoria, Australia; \(^3\)The University of Melbourne, Parkville, Victoria, Australia; \(^4\)Victorian Comprehensive Cancer Centre, Melbourne, Victoria, Australia; \(^5\)University of Cambridge; \(^6\)Queen Mary University of London and \(^7\)University of Sydney.

Body: Background: iPrevent estimates an individual's personal BC risk, using either the IBIS or BOADICEA algorithms, and provides tailored risk management information on screening, lifestyle modifications, risk-reducing surgery and risk-reducing medication. It is designed to be used collaboratively by women and their clinicians. The purpose of this pre-implementation pilot study was to assess the clinical usability and acceptability of the iPrevent prototype, and to identify barriers to clinical implementation. Exploratory aims investigated patients' BC worry, anxiety, risk perception and knowledge before and after using iPrevent. Methods: Eligible clinicians worked in primary care (PC), breast surgical (BS) or genetics clinics (GC). Their female patients were eligible if aged 18-70 years with no personal cancer history. Clinicians were familiarized with iPrevent using hypothetical cases, then actor scenarios, and lastly iPrevent was trialed with patients. All participants completed the System Usability Scale (SUS) and an acceptability questionnaire 2 weeks after using iPrevent. Patients also completed the Lerman BC Worry Scale, Spielberger State-Trait Anxiety Inventory, and BC risk perception and prevention knowledge questionnaires before and 2 weeks after using the tool. Data were summarized using descriptive statistics. Results: 63 participants comprising 20 clinicians (median age 47 years, 8 PC, 6 BS, 6 GC) and 43 patients (median age 38 years, 16% high risk, 51% moderate risk, 33% average risk) were recruited. Usability was rated above average (SUS score >68) by most clinicians (68%) and patients (76%). Most (79% of clinicians, 81% of patients) agreed iPrevent was ‘easy to use’, although 10 (53%) clinicians and 10 (27%) patients reported that it was too long. Most clinicians (84%) and patients (86%) found iPrevent ‘very’ or ‘somewhat’ helpful. 89% of participants reported that iPrevent provided the right amount of information. 5% reported to ‘rarely’ or ‘not at all’ worry about BC before iPrevent, and 29% after use. 25% of patients reported less impact of worrying about BC after iPrevent, 47% were unchanged and 28% reported more impact of worrying about BC after iPrevent use. State anxiety remained the same. 87% of patients correctly reported their risk category after using iPrevent\(^\circ\) compared with 40% before. BC prevention knowledge improved for most questions. Conclusions: iPrevent has high usability and acceptability. Exploratory analyses suggest that iPrevent may also improve patients’ BC risk perception and knowledge without adversely affecting anxiety or BC worry. Because concerns about length could be a barrier to implementation, data entry has been abbreviated in the modified version of iPrevent that will be publically available.
**Title:** Reducing overtreatment of ductal carcinoma in situ through active surveillance: Harm-benefit tradeoffs from the patient perspective

Marc D Ryser¹,², Yiling Liu³, Laura Hendrix², Terry Hyslop³ and E Shelley Hwang¹. ¹Duke University Medical Center, Durham, NC; ²Duke University, Durham, NC and ³Duke University Medical Center, Durham, NC.

**Body: Background.** Every year, over 60,000 women in the US are diagnosed with ductal carcinoma in situ (DCIS), a precursor lesion of invasive breast cancer. Despite a low risk of progression to invasive disease, guideline concordant care (GCC) for DCIS patients consists of invasive surgery and radiation. To mitigate the resulting overtreatment and associated harms, ongoing trials are evaluating active surveillance (AS) as an alternative management strategy for patients diagnosed with low-risk DCIS. In practice, clinical implementation of AS will require careful assessment of the harm-benefit tradeoffs compared to GCC. Therefore, our objective was to quantify these tradeoffs in a patient-centered framework.

**Methods.** The harm-benefit tradeoffs were quantified using multiple outcome measures: invasive disease-free survival, risk of future mastectomy, risk of surgery-associated harms, disease-specific mortality, and other cause mortality. Data from SEER-Medicare, the National Cancer Database, and relevant observational studies and clinical trials were integrated in a Bayesian evidence synthesis framework. Mathematical models were developed to predict patient-specific risks for competing management strategies. Probabilistic willingness-to-pay analyses were used to identify optimal management strategies based on patient-specific risk tolerance levels and multivariable harm-benefit measures.

**Results.** Differential risk profiling revealed substantial differences between AS and GCC with respect to invasive disease-free survival, risk of future mastectomy and surgery-associated harms, but less so with respect to disease-specific mortality. Other cause mortality varied considerably with patient age and comorbidity status at diagnosis. Personal risk tolerance played a critical role in identifying acceptable patient-specific tradeoffs of competing management strategies.

**Conclusion.** The harm-benefit tradeoffs between GCC and AS strategies for patients diagnosed with DCIS are complex. Because the tradeoffs critically depend on patient characteristics and risk tolerance levels, informed decision making requires effective communication of personalized risk projections. These findings emphasize the need for patient-tailored decision support tools as a critical first step in mitigating overtreatment of DCIS through active surveillance.
Title: Talking about risk in the context of GEomic profiling tests (TARGET)

Lesley J Fallowfield and Valerie A Jenkins. SHORE-C, BSMS, University of Sussex, Brighton, United Kingdom.

**Body:**

**Background:** Risk of recurrence scores from genomic profiling tests such as OncotypeDX® and EndoPredict® are being used increasingly with other clinico-pathologic features to help determine the likely benefit of adjuvant chemotherapy in early stage breast cancer. Decision-making requires the balancing of likely absolute benefits in terms of preventing recurrence versus the treatment related side effects. Health literacy and numeracy skills in the general population are often poor thus explaining risk and uncertainty can be confusing especially when set against a backdrop of fear and anxiety. As clinicians are facing more of these types of conversations with their patients we developed an educational program to help when discussing genomic test results.

**Methods:** The development of the educational package followed discussions with key clinicians who routinely used genomic profiling tests, clinician-scientists and a review of the risk literature. We mapped out the difficulties they encountered when explaining high, intermediate and low risk test results together with the added challenges faced when communicating with patients with different personality and socio-educational characteristics. As clinicians and their patients may both have a measurable intolerance to uncertainty this may contribute irrespective of the recurrence score (RS) to seemingly irrational decisions about treatment. We rehearsed simulated patients (actors) experienced in improvisation to create different characters and filmed unscripted genomic test result consultations with cancer clinicians. This process proved successful in previous educational initiatives aimed at improving communication about clinical trials. (Jenkins et al 2006; Fallowfield et al 2012).

**Results:** The educational package comprises an interview with Professor Mitch Dowsett explaining the science behind gene expression profiling tests, a lecture on the psychology of risk with group exercises and strategies on how to communicate together with 5 filmed scenarios with a timecoded facilitator handbook. The scenarios depicted are based on real clinical situations and demonstrate some of the issues discussing RS with low risk patients who nevertheless wish to have chemotherapy as well as high risk patients who are averse to chemotherapy.

**Conclusion:** Discussions about the logic and rationale behind different treatment recommendations for breast cancer have become increasingly complex. Clinicians need an increased repertoire of communication skills to explain risks and benefits. We are now evaluating the efficacy of TARGET prior to training facilitators to roll the program out.
Improving neoadjuvant breast cancer therapy rates uptake with education and technology

Monique Gary¹, Vickie Keeler¹, Suzanne Rush¹, Pat Parsons¹, Xin Zhong², Carrie T Stricker³, Debra Wujcik³, Laura DiGiovanni³, Agnes Davis³ and Linda K Han⁴. ¹Grand View Health, Sellersville, PA; ²Indiana University Health, Avon, IN; ³Carevive Systems, Inc., North Miami, FL and ⁴Parkview Physicians Group, Fort Wayne, IN.

**Body: Background:** Recent studies show that treating aggressive subtypes of breast cancer (BC) with neoadjuvant chemotherapy (NAC) improves clinical outcomes in addition to breast conservation therapy (BCT) rates. Yet a large multi-site population-level analysis shows that only 5.5% of NCCN-guideline eligible patients receive NAC (Ontilo et al, 2013). Multi-level interventions are needed to improve concordance with NCCN guidelines for NAC consideration in women who meet criteria for BCT (clinical stage IIA, IIB, and IIIA BC).

**Methods:** A 2-part intervention was undertaken to improve adherence to NAC guidelines. Certified medical education (CME) was first provided on BC diagnostics and treatment (Tx), including NAC. Next patients were recruited to a point of care technology-based intervention. Eligibility included a new diagnosis of invasive BC, clinical stage T1c and/or N1 or greater, and no prior Tx. Patients interact with an electronic care planning system (CPS) at the time of surgical consultation to report preferences for decision-making and concerns, such as distress over losing a breast. The CPS displays these findings along with a draft care plan (CP) that suggests guideline based referrals and provides patient education about BC diagnosis and Tx options. After editing, surgeons finalize and deliver CPs at the visit. The goal is to describe referral rates to medical oncology for discussion of and receipt of NAC. Outcomes from chart abstraction are compared to historical rates in the literature and where available, the institution. **Results:** Data on 39 of 75 women are mature (remaining to be presented at meeting). Median age is 60 years (range 37-92) and clinical stage is IA=41% (N=16), IIA= 41% (N=16), IIB=8% (N=3), and III=10% (N=4). Of 39 patients, 44% were HR+HER2+, 10% were HR+HER2-, 13% had triple negative BC, and 33% had incomplete data. Per NCCN stage, 59% (N=23) were eligible for NAC evaluation. 96% (N=22) of those eligible were referred to MO. Follow up 2 months post-surgical appointment revealed 91% (N=21) of referred patients had completed a MO consultation. 39% (N=9) of those referred for evaluation (N=23) had a prescription for NAC and all prescriptions were guideline adherent, including regimens combining chemotherapy with trastuzumab and pertuzumab for HER2+ disease. Overall, 30.4% of women eligible for referral went on to receive NAC. Distress related to loss of breast was moderate (0-10 scale, M=4.83) and was significantly related to whether patients received a referral for NAC (B= -.304, Wald's=4.61, p=.03). Most of participating providers (80%, N=5) felt the CP was valuable to help with Tx decision-making. **Conclusions:** Preliminary results show CME and an electronic CPS may improve NAC uptake. Rates of prescription were clearly higher in this analysis than in a 4-center population database study, both overall (23.1% vs. 3.8%) and by NCCN eligibility (30.4% vs. 5.5%), and compared to baseline in 1 (of 3 planned) centers in the study who had a baseline rate of overall NAC prescription of 8.7% in the year prior to the study. The higher the distress over the loss of a breast, the more likely the patient received a referral for NAC. These data provide preliminary support for improving NAC uptake and warrant investigation in a RCT.
Title: The role of patient perceptions in under reporting chemotherapy induced peripheral neuropathy (CIPN)

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Body: Background: Although paclitaxel remains one of the most efficacious and commonly used agents in the treatment of breast cancer, it can cause a number of side effects. Decisions to delay, decrease, or discontinue treatment are made based on the severity of chemotherapy induced peripheral neuropathy (CIPN), which is not objectively measured but relies on patient's accurately reporting symptoms to their clinical team. There is some concern that patients do not accurately or completely report CIPN symptoms during treatment. A previous study reported that 3 of 24 patients (12.5%) considered or could understand another patient's decision to under-report CIPN symptoms to avoid treatment disruption. The objective of this follow-up study was to determine whether under-reporting occurred and to understand patient perspectives on topics previously found relevant to a patient's decision to under-report CIPN.

Methods: Ten patients with early stage breast cancer who received adjuvant paclitaxel 80 mg/m² for up to 12 weeks, who had no prior neuropathy or neurotoxic chemotherapy participated in a recorded, semi-structured, phone-based interview that followed an interview guide designed to cover topics relevant to patient under-reporting.

Themes identified in patient perceptions of objectives found relevant to under reporting

<table>
<thead>
<tr>
<th>Objective</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient Understanding</td>
<td>1. Patients had consistent descriptions of their expectations for CIPN prior to treatment. 2. Understanding of the long-term potential of CIPN varied among patients.</td>
</tr>
<tr>
<td>Patient Education</td>
<td>1. Patients used a variety of sources to find information about CIPN, with the most commonly used sources being medical staff and patient handouts.</td>
</tr>
<tr>
<td>Patient Input</td>
<td>1. Patients agree on the importance of reporting side effects, but not to what extent. 2. Patients felt included in the treatment decision-making process, but some felt the doctor ultimately made the decision. 3. Providers may plan an important role in the extent to which patients report their symptoms.</td>
</tr>
<tr>
<td>Treatment Cycles</td>
<td>1. Patients recognized that they may not complete all 12 cycles, but were determined to do so.</td>
</tr>
<tr>
<td>Perception Changes</td>
<td>1. Before treatment, patients are much more focused on efficacy and were not particularly concerned with side effects. 2. During treatment, patients reflected on their desire to complete treatment and the tolerability of the side effects they were experiencing. 3. After treatment, patients felt they should have asked more questions before starting treatment.</td>
</tr>
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</table>

The interviews were then transcribed and qualitatively analyzed using NVivo software to identify common themes.

Results: No patients in this study admitted to under-reporting neuropathy. Themes that emerged from the interviews are presented in Table 1.

Conclusion: Insight as to how patients perceive various aspects of paclitaxel treatment revealed that comfort level with providers, inclusion in the decision-making process, and encouragement to fully disclose all symptoms played an important role when patients considered whether to report adverse effects. Future research may focus on whether a lack of health literacy and/or health care related work experience predisposes to CIPN under-reporting.
Title: Shared decision making (SDM) in routine care treatment of breast cancer patients – a survey of patients following surgery

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Body: Introduction: The aim of shared decision making (SDM), defined as an interaction between patient and attending physician(s), is a treatment decision in which patients are meaningfully involved. Based on mutual agreement and active participation the awareness of a choice should be created and the choice respected. Many preference-sensitive decisions have to be made in breast cancer treatment. However, little is known about the implementation of SDM in German breast cancer care. We therefore investigated the process of SDM from the patients' perspective.

Methods: All breast cancer patients who underwent surgery in one of four certified breast cancer centers in Germany between 07/2016 and 12/2016 were invited by mail to participate in the survey. The experienced decision-making process was assessed using the 9-item Shared Decision Making Questionnaire (SDM-Q-9). SDM-Q-9 items were rated on a 6-point scale ranging from "completely disagree" to "completely agree", added together and transformed into a scale ranging from 0 to 100. The higher the total score the higher the experienced degree of participation in the decision-making process. The survey also assessed patients' satisfaction with treatment, satisfaction with decisions and decisional control preferences, and included a range of demographic and clinical questions. For most items we asked the participants to separately rate decision-making consultations with their inpatient hospital doctors, outpatient gynecologists, outpatient oncologists and primary care providers (PCP). The project is still ongoing, data of approximately 300 patients will be presented at the meeting in December.

Results: Of 289 patients approached by mail, 143 filled in the survey (response rate: 49%). Median age at the time of the survey was 62 years (36-89). 83% had breast conserving surgery, 17% mastectomy. 74% were treated with radiation, 31% received neo-/adjuvant chemotherapy. 14% were off-treatment at the time of survey participation, 67% still received antihormonal therapy, 9% anti HER2 treatment, 7% chemotherapy and 2% radiation. Inpatient hospital doctors achieved the highest SDM-Q-9 score (mean of 75, standard deviation of 22) indicating the highest degree of SDM. Oncologists, gynecologists and PCP were rated quite comparable with a mean score of 72 each and standard deviations (SD) of 27, 22 and 31. The mean score for all groups of doctors was 73. For items concerning satisfaction with quality and amount of doctors’ information and participation in medical decisions patients showed a high degree of satisfaction, resulting in mean values of 3.6 and 3.5 with SD of 0.6 on a 4-point scale ranging from "1" "very unsatisfied" to "4" "very satisfied".

Conclusions: A considerable number of patients took part in the survey. Overall, patients reported to have experienced SDM in many situations where treatment decisions were necessary. Patients were quite satisfied with the quality of information and their participation in medical decisions. However, we do not know whether non-respondents might have had different experiences regarding their treatment decision-making. Further research should include SDM expert observations of breast cancer treatment decisions to validate these findings.
Body: Rationale: The 70-gene signature (MammaPrint®, MP) is a prognostic test which guides treatment decisions in patients with early breast cancer. After level 1A evidence for clinical utility of MP has been proven, cost-effectiveness data is important to inform reimbursement.

Research objectives: To compare cost-effectiveness of adding MP to clinical risk assessment versus clinical risk assessment alone for the US and EU. Clinical risk was assessed by Adjuvant Online! (AOL) as described in Cardoso et al. NEJM 2016. We used prospective survival data from the large randomized phase 3 trial 'Microarray In Node-negative and 1 to 3 positive lymph node Disease may Avoid ChemoTherapy' (MINDACT).

Methods: We used a Markov decision model to estimate the expected costs and outcomes (quality adjusted life years; QALYs) for MP versus AOL in early breast cancer patients from a payers perspective in the US and a societal perspective in the EU over a 5 year time horizon. Five year breast cancer overall- and distant metastasis free survival was calculated based on the MINDACT population (n=6,693). Utility scores were collected by means of the EuroQol-5D in the pilot phase of MINDACT (the first n=800 MINDACT patients). Cost data were for the US based on published insurance claim data, for the EU on published health care- and societal costs. The cost-effectiveness was calculated for: (1) total early stage breast cancer population, (2) clinical high risk population and (3) clinical high risk group in the ER+/HER2- population. Finally, budget impact for a high-low range of different countries was calculated, as the application and costs of chemotherapy can be highly variable between countries.

Results: For all groups (1,2,and 3) in the US, using MINDACT survival data and insurance claim data, adding MP to AOL saved costs and gained more QALYs compared to AOL alone (total costs per patient $42,223 vs $45,566 and 4.035 vs 4.031 QALYs respectively). Thus, a small difference in quality adjusted life years (0.0041) was observed, whereas a large difference in costs ($3,342) renders MP a highly cost-effective test (less costly & more effective in 64% of the cases). The largest cost-benefit effect was seen for group 3. The cut-off point for MP being cost-effective in the total population (group 1) is when the chemotherapy costs (and consequences) together are above $30,000. In the US, with approximately 250,000 new breast cancer patients per year, and a cost saving of $3,342, annual budget savings are expected to be $836M. Similar results for the Netherlands (15,000 breast cancer patients per year), reveal a cost difference of $300 per patient, and overall annual budget savings are expected to be $4.5M.

Conclusion: Adding MP to clinical risk assessment is highly cost-effective compared to clinical risk assessment alone, based on the MINDACT survival data and US insurance claim data, for all above mentioned groups. When costs for chemotherapy (and consequences) exceed $30,000, MP is cost-effective for the total early breast cancer population. When costs for chemotherapy (and consequences) are below $30,000, the MP is cost-effective for the clinical high risk early breast cancer group. The separate results for EU countries will follow.
Cost-effectiveness of genetic testing with a hereditary cancer panel in women at risk of hereditary breast cancer

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Background: The role of the BRCA1/2 genes in breast cancer risk has been well characterized. However, pathogenic variants in additional genes also confer hereditary breast cancer risk and if detected can lead to interventions that can facilitate increased life expectancy. For example, the National Comprehensive Cancer Network (NCCN) guidelines (Version 2.2017) recommend MRI screening for women who carry a pathogenic variant in CHEK2 and MRI screening or risk-reducing mastectomy for women who carry a pathogenic variant in PALB2. Testing women at risk of hereditary breast cancer with a 7-gene panel was recently reported to be more cost-effective than testing only BRCA1/2 genes (Li et al. Value Health, 2017).

Objectives: To evaluate the cost-effectiveness of a multigene panel testing strategy including 11 genes with risk reduction recommendations by the NCCN guidelines compared with testing only BRCA1/2 genes.

Methods: Using a Markov model modified from the recent Li et al. publication, the 11-gene model was used to estimate life expectancies and total healthcare costs from a payers' perspective. The model included costs for genetic testing, genetic consultation, and cancer treatment as well as risk reduction strategies recommended by the guidelines: prophylactic surgery, MRI surveillance, and mammography. Probabilistic sensitivity analyses were carried out to determine the robustness of the modeling.

Results: In the base case, the incremental cost-effectiveness ratio (ICER) for the 11-gene multigene testing strategy compared with BRCA1/2 testing was $22,377 per life-year gained ($47,240 per quality-adjusted life-year gained) for a 40-year-old cohort and $36,690 per life-year gained ($63,384 per quality-adjusted life-year gained) for a 50-year-old cohort. The sensitivity analysis indicated that the multigene testing strategy had a 97% chance of costing less than $100,000 per quality-adjusted life-year gained for the 40-year-old cohort.

Conclusion: In patients at risk of hereditary breast cancer, testing using a multigene panel followed by risk reducing interventions is cost-effective compared with testing only BRCA1/2 genes.
Body: Introduction:
Health care spending rose from 5% to 17.8% of GDP between 1960 and 2015. Clinicians and researchers must engage in increasing health care value – better outcomes at less cost. Personalized screening is one such opportunity. The Patient Centered Outcomes Research Institute recently funded WISDOM (Women Informed to Screen Depending On Measures of risk), a randomized trial to tests the safety and efficacy of basing starting age, stopping age, frequency and modality of breast cancer screening on individual risk (Clinical Trials Identifier NCT02620852). The personalized arm of WISDOM integrates genetic testing into the risk algorithm. Funding for the clinical services of WISDOM (genetic test, risk assessment, high-risk counseling) are expected to be covered (health plans, insurers). Risk determines the frequency, time to initiate screening and drives cost of downstream screening services. The cost of genetic testing is now less than $250, comparable to a mammogram. The WISDOM study model brings payers, policy makers, provider, technology, and advocate partners together to generate evidence to see if risk based screening is as safe, less morbid, preferred by women, promotes prevention, and has greater health care value. Health plans need to know the value proposition, thus we evaluated financial implications of coverage for risk-based screening.

Methods:
A model was developed to compare costs and benefits of risk-based vs. current screening practices from the perspective of a health plan. Modeled cohorts resembled a screening population with risk determining screening interval for the risk-based model, and average time between mammograms determining the interval for the model of current screening practices. Model parameters were gathered from published literature, national databases, early findings from WISDOM and health plan claims data. Sensitivity analysis was performed on all parameters, including costs of clinical services, screening rates, and health plan turnover. The clinical services specific to WISDOM use a fixed-fee schedule, and not varied in the model. All other costs were conservative, based on Medicare rates and published literature.

Results:
We estimated that over five years, risk-based screening is at worst cost neutral with potential for savings of up to $215 per participant. Based on current trial enrollment, we estimated that 30 per 1,000 health plan enrollees would join, resulting in an upfront cost of $6,000 for WISDOM-specific services, primarily the genetic test, and $600 in ongoing costs after Year 1. However, the health plan would save on mammogram and work up costs as participants would receive an average of 2-3 fewer mammograms over five years. Savings are sensitive to the age of participants, cost of mammograms, and savings increase over time. Per participant, five-year savings of $300 and $35 for those aged 40-49 and 65-74 respectively, and increased costs of $30 for those aged 50-64. Overall, an upfront investment of $6,000 per 1,000 health plan enrollees (30 participants) yields $3,800 in five-year savings.

Conclusion:
Personalized screening could provide cost savings and has the potential to increase health care value. Enrollment in the Wisdom study is ongoing and results will be reported in 5 years.
Cost-effectiveness analysis of locally advanced estrogen receptor-positive, HER-2 negative breast cancer care using a tailored treatment approach in Brazil

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Body: Introduction: Breast cancer is the most common cancer in women worldwide, and 70% of breast cancer deaths occur in women from low-income and middle-income countries. In Brazil there were 14388 deaths due to this disease in 2013 and an estimate of over 58000 new cases in 2016. Neoadjuvant endocrine therapy (NET) is an attractive alternative to Neoadjuvant chemotherapy (NAC) for Hormone Receptor-positive tumors and could be a resources-saving strategy of treatment.

Methods: We built a decision analysis model of breast cancer treatment to compare a NET schema, with response based on the evaluation of Ki-67, against the surgery followed by adjuvant chemotherapy (AC) and radiation therapy (RT) standard-of-care as two competing approaches to breast cancer management. Our objective is to determine whether tailoring chemotherapy treatment based on response to neoadjuvant endocrine therapy is a cost-effective approach. The NET schema is based on the ACOSOG Z1031B trial, in which post-menopausal women with estrogen receptor-positive, HER-2 negative disease would receive 4 weeks of NET followed by a core-needle biopsy for Ki-67 evaluation. If Ki-67 were lower than 10%, patients would continue in NET for 16-18 weeks followed by surgery and RT according to international guidelines. The indication of AC in these patients would be based on the preoperative endocrine prognostic index (PEPI). Patients with a PEPI score equal to zero would be spared from AC. If Ki-67>10%, patients would be triaged to NAC or surgery. The cost-effectiveness analysis was conducted using a Markov model from the provider's perspective, in this case the Brazilian Health ministry. Healthcare costs, in the form of charges from the hospitals to the health ministry, were obtained from cost tables available at the federal government's webpage. In the Markov model, possible health states were disease-free, local relapse, metastatic disease and death. Transition probabilities and mortality rates were extracted from randomized studies. Our assumptions were that both treatment strategies have similar clinical outcomes and that Ki-67 is a reliable method to triage patients to NAC or surgery. We performed one-way sensitivity analysis to assess the impact of the failure of the Ki-67 test on cost-effectiveness.

Results: Our model shows that the NET schema dominates the standard-of-care strategy. Costs were R$ 47799.89 per patient for the NET strategy and R$79809.24 for the standard-of-care strategy. There was an incremental cost saving of R$32009.36 per patient for the NET strategy compared to the standard-of-care strategy. Cost-effectiveness of the NET strategy was R$2612.63 and R$4369.11 for the standard-of-care. Considering the willingness-to-pay of R$ 85494.00, defined by the World Health Organization as three times the gross domestic product per capita, the standard-of-care strategy would only be more cost-effective in the scenario of a Ki-67 test that misclassifies patients more than 9.1% of the time.

Conclusion: The use of response to neoadjuvant endocrine treatment based on Ki-67 analysis as a way to tailor locally advanced breast cancer treatment is a cost-saving strategy in the presence of robust biomarkers.
2017 San Antonio Breast Cancer Symposium

Publication Number: P4-12-05

Title: Cost-effectiveness analysis of second-generation multi-gene expression prognostic assays compared with the standard 21-gene recurrence score assay to guide adjuvant therapy decisions in women with early stage breast cancer

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Body: Second-generation multi-gene expression assays can generate comprehensive molecular risk scores which may inform adjuvant chemotherapy decisions in women with early breast cancer. The 12-gene EPclin score assay (EndoPredict®) and 50-gene PAM50-risk of recurrence (ROR) score assay (Prosigna®) appeared to have better prognostic value when compared to the current north American standard 21-gene recurrence score (RS) assay (Oncotype DX®). We sought to investigate the cost-effectiveness of using EPclin and ROR score assays versus RS assay in women with axillary lymph node-negative (LN−), hormone receptor–positive (HR+), and human epidermal growth factor receptor 2–negative (HER2−) early-stage operable breast cancer (ESBC) from the perspective of the Canadian public healthcare system.

We developed a Markov model to project the lifetime clinical and economic consequences of operable LN− HR+ HER2− ESBC. We assumed that women within each risk category by RS assay (low, intermediate and high) would be reclassified to binary risk categories (low and high) by EPclin score assay and to three risk categories (low, intermediate and high) by ROR score assay. The decision model was parameterized using 10-year follow up data from retrospective analyses of the Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial, cost data from the London Regional Cancer Program (Ontario, Canada) and secondary sources. Costs are presented in 2017 Canadian dollars. Future costs and benefits were discounted at 5%.

EPclin and ROR score-based strategies led to an increase of 0.04 and 0.02 quality adjusted life years (QALY)/person and a decrease in cost of $917 and $600/person respectively, resulting in both strategies being cost-saving compared to RS-based strategy. Incorporating the EPclin and ROR score assays in place of the current standard RS-assay for operable LN− HR+ HER2− ESBC patients in Canada would result in total gains of 469 and 250 QALYs/year and total savings of $11.5 and $7.5 million/year, respectively. EPclin compared to ROR score-based strategy led to an increase of 0.02 QALY/person and a decrease in cost of $317/person, resulting in EPclin score-based strategy being dominant. Our results were most sensitive to the proportion of women classified by EPclin and ROR score assays to different risk categories and who received adjuvant chemotherapy. A value-of-information analysis revealed that the total expected value of perfect information about the EPclin and ROR score assays' clinical impact was $95 and $55 million/year, respectively.

Our results indicate that the EPclin and ROR score assays are both clinically and economically attractive for patients with operable LN− HR+ HER2− ESBC in the Canadian healthcare setting. Both assays should be considered for adoption in place of the current standard RS-assay for this patient population. The EPclin compared to the ROR score assay appears to be clinically more promising and provides greater value for money in the Canadian healthcare system. Field evaluations of the EPclin and ROR score assays in real-world Canadian clinical practice are associated with a large societal benefit and warranted.
Title: Cost-effectiveness analysis of pertuzumab plus trastuzumab for advanced HER2-positive breast cancer in Brazil: A public health system perspective

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Body: Objectives:
Addition of pertuzumab to trastuzumab and docetaxel (THP) results in significant clinical benefits for women with HER2-positive metastatic breast cancer. Despite evidence of important overall survival and quality of life improvements, for the Brazilian patients depending on the public health system chemotherapy alone (CT) remains the standard treatment. Previous request for trastuzumab incorporation was denied by Brazilian Ministry of Health (MoH) due to high individual costs. In an innovative way, the Brazilian Society of Clinical Oncology made a price negotiation directly with the pharmaceutical company, aiming to propose a formal request for the incorporation of these technologies to the National Committee for Health Technology Incorporation (CONITEC).

Our objective was to evaluate the cost-effectiveness of THP versus CT in patients with HER2-positive advanced breast cancer, under the perspective of the Brazilian public health system.

Methods:
A cost-effectiveness analysis was conducted using a Markov model over a lifetime horizon. The model considers three health states: 1st-line treatment without progression; disease progression (with chemotherapy or best supportive care) and death. Transition probabilities and mortality rates were extracted from randomized studies. Costs of standard chemotherapy, complications, and surveillance were obtained from price tables regulated by the MoH. Pertuzumab and trastuzumab prices were those negotiated with the manufacturing company. Benefits are presented in life-years (LY) and costs in USD (using exchange rate for Brazilian Real to USD = 3.50). The relation between costs and benefits were used to present the incremental cost-effectiveness ratio (ICER) per life-year saved. We have performed one-way deterministic sensitivity analyses with cost of monoclonal antibodies to define the ideal price in Brazil.

Results:
Patients with HER2-positive metastatic breast cancer treated with CT were projected to have 1.87 LY, with a total cost of $12,118. THP was projected to increase the life expectancy of these patients by 1.48 years, with an incremental cost of $63,685 per patient. Thus, the ICER was $42,893 per LY-gained.

Conclusions:
The World Health Organization recommendation of three times gross domestic product per capita have defined our cost-effectiveness threshold. Despite the discount price, at a willingness-to-pay (WTP) threshold of $25,615 per LY, THP is not a cost-effective strategy compared to CT. An additional reduction around 58.67% in the pertuzumab price would be necessary to make THP cost-effective in Brazil.

Discussion about the incorporation of trastuzumab to CT using the discount prices is in progress.
Title: Cost-minimization of chemotherapy-induced (febrile) neutropenia prophylaxis with biosimilar ZARXIO® over NEUPOGEN®, NEULASTA®, and NEULASTA/ONPRO®: Breast cancer case study

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Body: RATIONALE & OBJECTIVES: Biosimilar filgrastim may offer significant cost advantages over originator filgrastim and pegfilgrastim. The objectives were (1) to evaluate for the US the comparative cost-minimization of chemotherapy-induced (febrile) neutropenia (CIN/FN) prophylaxis with biosimilar filgrastim ZARZIO® over originator filgrastim NEUPOGEN®, and originator pegfilgrastim NEULASTA® and NEULASTA/ONPRO® injection device with the health-care provider (HP) providing full administration, using 3Q2016 average selling price (ASP); and (2) to apply the different savings estimates to a breast cancer case study.

METHODS: Cost-minimization analysis of [1] acquisition costs for one patient for one chemotherapy cycle for 1 to 14 days (d) using per unit dose, and [2] administration costs using Current Procedural Terminology (CPT) codes. We calculated [1] the general cost of prophylaxis for one cycle with each agent, with standard filgrastim administrations ranging from 1-14 days and pegfilgrastim limited to single administration; and [2] the cost-savings that could be accrued from 1-14d prophylaxis with ZARXIO® over the three originator options. The case study concerns a 43 y/o Caucasian female, newly diagnosed with stage 2 HER2-negative breast cancer being started on TAC (FN risk >20%); unremarkable medical history; no comorbidities; with primary prophylaxis initiated in cycle 1 and continued through 6 cycles per local protocol (single NEULASTA® or NEULASTA/ONPRO® or 11d NEUPOGEN® or ZARXIO®).

RESULTS: Using ASP+CPT, prophylaxis cost per dose (rounded) was $260 for ZARXIO®, $326 for NEUPOGEN®, $3,926 for NEULASTA®; $3,910 for NEULASTA®. In general, cost-savings per cycle from ZARXIO® over NEUPOGEN® ranged from $65 (1d) to $916 (14d); over Neulasta®, from $3,666 (1d) to $284 (14d); and over NEULASTA/ONPRO®, from $3,649 (1d) to $267 (14d). In the breast cancer case study, cost of prophylaxis per one cycle was $2,862 for ZARXIO® (11d), $3,582 for NEUPOGEN® (11d) vs. $3926 for NEULASTA® and $3910 for NEULASTA/ONPRO® single-injection. Cost-savings per cycle from ZARXIO® use were $719 vs. NEUPOGEN®, $1,064 vs. NEULASTA®, and $1,047 vs. NEULASTA/ONPRO®. Total savings from ZARXIO® use over all 6 TAC cycles were $4,316 vs. NEUPOGEN®, $6,385 vs. NEULASTA®, and $6,284 vs. NEULASTA/ONPRO®.

CONCLUSIONS: In general, CIN/FN prophylaxis with ZARXIO® for 1-14d generates significant cost savings over NEUPOGEN®, NEULASTA® and NEULASTA/ONPRO® generating significant cost-savings. In the case study of the 43 y/o HER-negative breast cancer patient treated with TAC and prescribed 6 cycles of primary prophylaxis with 11d standard or single-administration pegfilgrastim, savings reached as high as $6,385 for the full course of chemotherapy. Given the trial evidence of non-inferiority of pegfilgrastim over filgrastim, the clinical trend for <14d of filgrastim prophylaxis, and payer trends to authorize filgrastim vs. pegfilgrastim prophylaxis, using biosimilar Zarxio® is rational from both a economic perspective; as illustrated also in the breast cancer case study.
Title: Palbociclib in hormone receptor positive advanced breast cancer: A cost-utility analysis

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Body: Introduction
The addition of palbociclib to letrozole improves progression free survival (PFS) and response rates compared to letrozole alone in the 1st line treatment of hormone receptor positive advanced breast cancer. However palbociclib increases toxicity (i.e. neutropenia) and costs more than $6,250 per month in Canada. This study assesses the cost-utility of palbociclib from the Canadian healthcare payer perspective.

Methods
To evaluate the cost-utility of palbociclib, a probabilistic discrete event simulation model was developed. The model was parameterized with data from the phase 2 and 3 PALOMA 1 and 2 trials and other sources. The incremental cost per quality-adjusted life-month (QALM) gained for palbociclib was calculated. A time horizon of 15 years was used in the base case with costs and effectiveness discounted 5% annually. The time to progression and death were derived from a Weibull and exponential distribution. Expected costs were based on Ontario fees and other sources. Probabilistic sensitivity analyses were conducted to account for parameter uncertainty.

Results
Compared to letrozole alone, the addition of palbociclib provided an additional 14.7 QALM at an incremental cost of $161,508. The resulting incremental cost-effectiveness ratio was $10,999 per QALM gained. Assuming a willingness to pay (WTP) of $4,167 per QALM, the addition of palbociclib was not cost-effective and the probability of palbociclib to be cost-effective was 0%. Cost-effectiveness acceptability curves derived from a probabilistic sensitivity analysis showed that at a WTP of $11,667 per QALM gained, the probability of palbociclib to be cost-effective was 50%.

Conclusion
Compared with letrozole alone, the addition of palbociclib is unlikely to be cost-effective for the treatment of advanced breast cancer from a Canadian healthcare perspective with its current price. While advanced breast cancer patients derive a meaningful clinical benefit from palbociclib, considerations should be given to increase the WTP threshold and reduce the drug pricing, to render this strategy more affordable.
Title: Molecular test for BRCA 1 and 2: Hereditary breast and ovarian cancer syndrome. Analysis of cost effectiveness of its implementation

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Body: Introduction
Breast cancer is the leading cause of women cancer. Hereditary cause represents 5% - 10% of cases. Molecular testing for BRCA1 and BRCA2 allows physicians to identify breast and ovarian cancer high risk women. Screening, diagnosis and treatment for high risk women differ from general population ones. As new drugs have been increasing health costs, the need of cost-effective strategies has raised. In Argentina, molecular tests are still very expensive and most health insurances do not cover it.

Objectives
To evaluate the long-term cost-effectiveness of germline BRCA1 and BRCA2 molecular testing in women with no personal history of cancer and >=10% mutation risk based on family history criteria, compared to no testing women.

Methods
We performed a decision-analytic model (decision tree) to compare the risks of breast and ovarian cancer, mortality rates, and costs and effects (life-years and QALYs) in women with and without molecular testing for BRCA1 and BRCA2. Carrier's probability undergoing risk-reducing surgeries was considered.
This data has been analized in two private hospitals of Buenos Aires, Argentina.

Results
BRCA testing in all women with >=10% mutation risk based on family history criteria resulted cost-effective and even cost-saving in our country, with an incremental cost-effectiveness ratio of -$315.353/QALY and -$1.357.143/life-year. Our results demonstrate to be robust after sensitivity analyses varying the input parameters.

Conclusion
BRCA testing is cost-saving in our country probably because of prophylactic risk-reducing surgeries in BRCA carriers. This leads to a reduction of breast and ovarian cancer cases.
Title: Evaluation of quality, cost, and value in clinical stage IA breast cancer

Tomas Dvorak1, Regan Rostorfer1, Jeffrey Smith1, Damien Coltey1, John Waters1 and Eleftherios Mamounas1. 1Orlando Health, Orlando, FL.

Body: Background: There is increasing emphasis in providing high-value care. Value can be interpreted as a ratio of quality of care delivered and the cost to provide that care. We set out to evaluate the value of our care by defining a set of quality metrics (points) for each patient, then evaluating our cost to the payors to deliver this care.

Methods: Patients with clinical Stage IA breast cancer managed completely at our Cancer Center between 1/1/2014 and 12/31/2014 were identified from cancer registry. An IRB-approved retrospective review of clinical charts and financial data was performed. Based on The Advisory Board Company metrics, a set of 18 quality measures was developed. These included process measures (time to initial biopsy, rate of needle biopsy, time to pathology reports, ER/PR and HER2 assessment, pathology synoptic report generation, time to surgery or neoadjuvant chemotherapy), treatment measures (performance of sentinel node biopsy, administration of chemotherapy for ER- or HER2+ disease, administration of radiation for lumpectomy or pN2/pN3 disease after mastectomy, administration of endocrine therapy for ER+ disease), and complication measures (flap complication after reconstruction, chemotherapy ER visits and inpatient admissions). Depending on the treatment pathway, patients were eligible for a different number of quality points. A patient received a quality point if they were eligible for the measure and met it. Financial review identified actual technical revenue received by the hospital, and apportioned it accordingly to the various revenue centers. Revenue was included for 365 days after the date of first contact, and was used as a proxy for cost to the payors. All patients were included regardless of type of insurance or free-care.

Results: There were 110 patients treated. All patients (100%) underwent surgery (lumpectomy 69%; mastectomy 5%, mastectomy with reconstruction 26%). Chemotherapy was delivered in 20% of patients (neoadjuvant 13%; adjuvant 7%). Radiation therapy was delivered in 57% of patients. Most common treatment pathways were lumpectomy with radiation (46%), mastectomy with reconstruction alone (18%), and lumpectomy alone (14%). Number of potential quality points depended on care pathway, and ranged from 6 to 15 per patient. There were 939 quality points achieved out of possible 1104 (85%) in the entire cohort. Quality ratios per patient varied from 55% to 100%. Lowest quality measure was time-to-surgery <=30 days at 75%. Overall revenue (cost to payors) was $6.2 million for the cohort. Medicare was 35% of patients. Average cost of care per patient was $56.5K (range $0 to $385K). The cost per point of quality was $6,443 (range $0 to $45.4K). Highest cost per quality point was in a commercial insurance patient treated with neoadjuvant TCHP, followed by bilateral mastectomy with DIEP reconstruction and radiation.

Conclusions: We have established a model to assess the value of breast cancer care provided as cost of care delivered per quality point achieved. To improve our value proposition to the payors, and ultimately to our patients, we plan to focus on improving our compliance with the quality measures, monitor care pathway utilization, and identify opportunities to lower the cost of care.
Title: Cost effectiveness of oncotype Dx for early stage breast cancer under National health insurance

Yumi Kim1 and Wonshik Han1,2. 1Seoul National University College of Medicine, Seoul, Korea and 2Cancer Research Institute, Seoul National University College of Medicine, Seoul, Korea.

Body: Background: Oncotype Dx is being used increasingly for risk stratification to identify for patients with estrogen receptor-positive (ER+), node negative early-stage breast cancer who are most likely to benefit from adjuvant chemotherapy. In Korea, all citizens are covered by national health insurance (NHI), besides patients just pay 5% of hospital bill, if who diagnosed with cancer. NHI can help solve the financial burden for cancer patients.

So the acquisition costs of the Oncotype DX test are sometimes a barrier to its widespread use. This study was conducted for existing cost-effectiveness analyses of Oncotype DX in Korea.

Methods: We analyzed the hospital expenses of patients who diagnosis with LN-negative ER-positive early-stage breast cancer from October 2010 to October 2016 in Seoul National University Hospital. All patients are possible candidates for adjuvant chemotherapy. Among them 273 patients were tested Oncotype Dx. A cost-utility analysis (CUA) was performed.

We analyzed the hospital expenses of chemotherapy by regimens who underwent adjuvant chemotherapy patients, and costs of accompanying adverse event. Each costs of therapy were derived from the hospital administration and expressed by USD. (KRW convert into USD) We also analyzed every patient charges and real expense.

Result:
Of 273 patients, 39(14.3%) were underwent adjuvant chemotherapy. The patients proportion by regimens are FAC(56.4%), AC(10.3%), AC followed by weekly paclitaxel (5.1%), TC(10.3%), AC followed by docetaxel (5.1%), TC and CMF etc. Each patient's hospital expense are roughly 3,000 USD (2,200 – 4,000 USD), it including all costs of laboratory test, computer tomography, chest x-ray, chemoagents and other medications. Of these costs, patients should pay only 5% so it could be around 150 USD (110-200USD).

Discussion:
The economic benefits of Oncotype DX are modelled by an overall reduction in chemotherapy usage, thus avoiding associated costs and disutility, and an increase in chemotherapy usage in the high RS group, reducing the risk of recurrence and improving health outcomes. Several studies shows Oncotype DX has been proposed to better estimate baseline risk and response to chemotherapy, to appropriately target chemotherapy to higher risk patients. In the costeffectiveness model, decision making with Oncotype DX in addition to standard decision-making tools is compared with standard decision making without Oncotype DX.

From this study, most patient (234 of 273, 85.7%) who are possible candidates for adjuvant chemotherapy avoiding the treatment. A number of studies have looked at the impact of Oncotype DX on clinical decision making. But under the special environment of Korea that most costs who diagnosed with cancer covered by National Insurance health, it relatively expensive than other continents. So it deserve much consideration that necessity of further studies and reaserches to develop new tools that could be more reasonable in cost in Korea.
Breast pain: A very common complaint - What workup is cost effective?

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Background: Mastalgia, without other signs or symptoms, is a very common complaint, yet occurs in only approximately 5% of patients with breast cancer. Most clinicians are aware of this lack of association with cancer. Mastalgia, however, remains worrisome to many women, and when seen in the office, clinicians tend to give in to the fear of patients and order various tests. Trusted surgical textbooks recommend that women 30 years and older with mastalgia undergo mammography and ultrasound, in addition to a physical examination, and other necessary workup. The current study was performed to evaluate the benefit and efficacy of working up patients with mastalgia.

Methods: The safety net hospital in Phoenix, Arizona serves a patient base which is 34.5% non-English speaking. The majority of these patients are uninsured or underinsured. A total of 7202 consecutive patients were seen at least once at the Breast Clinic from June 1, 2006 to February 28, 2017. Sociodemographic variables were collected on all patients. All patients who presented with a complaint of breast pain were evaluated to determine the efficacy of their workup. Additionally, all patients who complained of a breast mass but were found to have pain with no mass were evaluated in a similar fashion.

Results: More than 1 of 7, 15% of patients (1017 of 7202) presented with a complaint of breast pain. In addition, 702 patients who complained of a breast "mass" really were found to have only breast pain. Of the 1719 patients with breast pain, 15 (<1%) were found to have breast cancer. The average age of patients found to have cancer was 49 years. None of the patients found to have cancer were at increased risk according to Gail Model risk estimates (mean 5 yr risk 0.7% (range 0.2 – 1.4%). Only 1 patient (6%) were undergoing screening mammography. Physical examination identified a mass in 4 patients and the remaining cancers were found by mammogram. Of the 15 patients found to have breast cancer, most (13 of 15) were stage 0 or stage 1. Also only 1 patient had pain only on the side of the cancer.

A total of 880 patients were under the age of 40 years. Only 1 breast cancer patient was under age 40. 524 patients were between 40-49 years and 8 were found to have breast cancer. The remaining 315 patients were 50 years or older and 6 were found to have breast cancer.

Most patients underwent workup for their breast pain with 85% undergoing breast imaging (mammogram, ultrasound, or MRI, and even 2 CT scans), 32% undergoing a biopsy, and 68% having blood drawn for laboratory tests. To add to the costs, 27% of patients were seen in the Emergency Department prior to their visit at the Breast Clinic.

Conclusions: Mastalgia is a very common complaint in patients seen in outpatient clinics. In patients not recommended to undergo screening based on any National guideline (Age < 40 years), any workup beyond physical examination does not appear to be cost effective. In patients at least 40 years mammography should be performed if the patient has not been participating in routine screening. Diagnostic imaging (in the absence of findings on screening) and laboratory tests do not appear beneficial in patients with breast pain. Continued education is necessary to avoid continued use of unnecessary medical resources.
Comparative analysis of costs between subcutaneous formulation of trastuzumab versus intravenous formulation from the perspective of the Instituto Oncológico Nacional of Panamá from January to December 2016

Cristiane Martin¹, Juan Alcedo¹ and Erick Araúz¹. ¹Instituto Oncológico Nacional, Panamá, Panama.

Objective: Because there is actually availability of both Intravenous(IV) and subcutaneous(SC) formulation of Trastuzumab for use in the public sector in the Instituto Oncológico Nacional and knowing that de SC formulation has been compared previously with the IV in term of efficacy in and it was not inferior and also is prefered by patients it is useful to count with a study of costs minimization that can determine what formulation results more convenient to maintain available for use in the Instituto Oncológico Nacional based on the criteria of efficiency of the intervention.(1)(2)

Methodology: This is a cost minimization study considering the cost of the treatment, the supplies and the time utilized by Pharmacy and Nursing to prepare and administer it.

Results: In relation to the cost of the treatment the SC presentation has a cost per patient 17% less than the IV presentation in relation to the cost that offers the Pharmaceutical company, considering a weight of 70 kg per patient (IV presentation) and a fixed dose of 600 mg(SC presentation). Considering the supplies utilized by Pharmacy and Nursing to prepare and administer the IV and SC presentation, the SC presentation requires less supplies and this facilitates its administration and decreases the cost of the treatment in a 67%. In terms of time invested in preparing and administering the IV and the SC formulation, accounting for both, nursing and pharmacy, it is observed that the SC formulation requires less amount of time than the IV formulation and this decreases the cost of the treatment in a 90%.

Analisis of Saving by Use of SC Trastuzumab vs IV Trastuzumab

<table>
<thead>
<tr>
<th>Item</th>
<th>SC Trastuzumab</th>
<th>IV Trastuzumab</th>
<th>% Saving</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>10,578,053</td>
<td>8,826,894</td>
<td>17%</td>
</tr>
<tr>
<td>Nursing Supplies</td>
<td>18,966</td>
<td>2,503</td>
<td>87%</td>
</tr>
<tr>
<td>Pharmacy Supplies</td>
<td>31,982</td>
<td>17,020</td>
<td>47%</td>
</tr>
<tr>
<td>Nursing Time</td>
<td>71,791</td>
<td>6,335</td>
<td>91%</td>
</tr>
<tr>
<td>Pharmacy Time</td>
<td>2,289</td>
<td>716</td>
<td>69%</td>
</tr>
<tr>
<td>Total annual cost</td>
<td>10703081</td>
<td>8853467</td>
<td>17%</td>
</tr>
</tbody>
</table>

Conclusion: With the use of the SC formulation of Trastuzumab there is combined savings of 17%, that represents anually 5985 USD per patient based on a treatment of 18 cycles of Traztuzumab per year, that results in a total saving of 1,84 million USD per year.

(2)Ismael G et al. Lancet Oncol 2012 Sep;13(9):869-78
2017 San Antonio Breast Cancer Symposium

Publication Number: P4-13-01

Title: Oncoplastic breast conservations – The Scottish Audit: Surgical techniques, oncological outcomes, complication rates and variations in practice across the country based on the analysis of 589 patients

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Body: Introduction: current evidence for oncoplastic breast conservation (OBC) is based on single institutional series. We studied the outcomes of OBC practice in Scotland and compare individual breast units.

Methods: a predefined database of patients treated with OBC was completed retrospectively in 11 breast units in Scotland. Patients were treated with OBC from 2005 onwards were included. For statistical calculations Chi-test, ANOVA and Pearson correlation analysis were used.

Results: Altogether 589 patients were included. Median age was 56 years [21-86]. Patients were diagnosed between September 2005 and March 2017. Number of patients treated with OBC per unit ranged between 4 and 145. High volume units were doing a mean of 19.3 OBCs per year [17.3 – 26.5] vs. low volume units doing 11.1 OBCs per year [7.7– 14.4] (p=0.012).

23 different oncoplastic surgical techniques were applied. Range of oncoplastic techniques used was associated with case-loads: high volume units used a wider range (8 – 14 different techniques) compared to low volume units (3 – 6) (p=0.004). Volume displacement was done in 515 patients (91.3%), volume replacement in 49 patients (8.7%). OBC was carried out as a joint operation between a breast and a plastic surgeon in 66.3% (389 patients). Immediate contralateral symmetrisation rate was significantly higher when the procedure was carried out as a joint operation (70.7% vs. not joint operations: 29.8%; p<0.001).

Incomplete excision rate was 10.4% (60 of 578). Incomplete excision was significantly higher after invasive lobular carcinoma (18.9%; 10 of 43; p=0.0292). After neoadjuvant chemotherapy incomplete excision rate was significantly lower (3%; 2 of 66 vs. no neoadjuvant chemotherapy: 11%; 35 of 319; p=0.031).

Neoadjuvant systemic treatment rate was 28.6% (142 of 496 patients). Of those 68 patients received neoadjuvant chemotherapy (13.7%) and 74 patients had neoadjuvant hormonal treatment (14.9%). Neoadjuvant systemic treatment rate varied amongst the units from 9.7% to 57.2% for patients with invasive carcinoma.

259 patients diagnosed with (non)invasive carcinoma had a median follow-up time of 5 years [35-124]. Of these 7 patients (2.7%) developed isolated local recurrence. 5-year local recurrence rate after DCIS was higher than after pure invasive ductal carcinoma (DCIS: 8.3%; 3 of 36 vs. ductal: 1.6%; 3 of 181; p=0.02567). 5-year disease-free survival of these patients was 91.7%, overall survival was 93.8%, and cancer-specific survival was 96.1%.

145 of 510 patients developed complications, which is 28.4% overall complication rate. 71 patients had major complications (13.9%) and 74 patients had minor complications (14.5%). Overall complication rate was significantly lower after neoadjuvant chemotherapy (15.9%; 11 of 69) compared to patients who did not receive neoadjuvant chemotherapy (27.9%; 127 of 455 patients) (p=0.035).

Conclusion: this national audit demonstrated similar outcomes overall compared to relevant published data. Units should be urged to build stronger collaboration in order to reduce variability in OBC practices.

None of the authors have conflict of interest to declare.
2017 San Antonio Breast Cancer Symposium

Publication Number: P4-13-02

Title: Exclusive fat grafting breast reconstruction after mastectomy: Aesthetic results, satisfaction and quality of life evaluation on 38 patients

Virginie Bordes¹, Mathilde Simorre², Loïc Campion¹, Florence Lejeune³, Yves Loirat³, François Dravet¹ and Anne-Laure Bouffaut³. ¹ICO René Gauducheau, Nantes, France; ²CH, Saint Nazaire, France and ³Clinique Breteche, Nantes, France.

Body: Background: Autologous fat grafting has become a frequent, simply reproducible and low-risk technique in breast reconstruction. The potential risk of fat tissue transfer to the breast for oncologic patients remains to be discussed, but one must clearly distinguish the situation where there is a breast parenchyma left and where the whole gland has been removed, like in our study. Although lipotransfer has become very popular, only a limited number of case series have been reported up to date. The presented study evaluates aesthetic results and quality of life after exclusive fat grafting breast reconstruction.

Patients and methods: A retrospective study was performed in two French centers with five surgeons between February 2011 and June 2015. We included patients with prior breast cancer, treated by mastectomy and with a finished breast reconstruction with exclusive fat grafting. We excluded patients with implant or flap. For each patient, the aesthetic evaluation was threefold, performed by the patient, the surgeon and an extra person, using the same questionnaire. For the analysis of the cosmetic results, the patients, surgeons and the other person were asked to grade the result on a 0 to 10 scale, ranging from "very bad" to "very good". They were questioned about the global esthetic result, symmetry between the two breasts and reconstructed breast texture. Satisfaction was evaluated using a Breast-Q adapted questionnaire, elaborated by psycho-oncologists and surgeons. Quality of life was evaluated using WHOQOL-BREF 26. Statistical analysis was performed using stata 13.1 SE.

Results: We sent a questionnaire to 48 patients and we obtained 38 responses. The mean age of the patients was 52 years, 31 patients (81,6%) lived in couple and 29 patients (76,3%) were employed. We performed 190 fat grafting procedures with an average of 4,2 per patient. The mean total quantity of fat injected was 904 ml per patient with a mean quantity per procedure of 219 ml. The mean time between two procedures was 4,3 months. The average grade obtained for the global esthetic result was 7,3 +/- 1,8 out of 10 for patients, 7,6 +/- 2 for the extra person and 7,9 +/- 1,4 for surgeons. For symmetry between the two breasts, the result was 7,1 +/- 1,9 and for the texture, it was 6,8 +/- 2,6. To the question "did the final result meet your expectations?" 81,5% (31/38) of the patients and 79% (30/38) of the surgeons said yes. Among the 29 patients having a professional activity, 86% (25/29) of patients were able to work between each fat grafting session. The handicap evaluation in the professional life for these patients from 0 "no handicap" to 10 "important handicap" showed a score of 2,24 +/-2,7. As for global quality of life evaluation, to the question: "how would you grade your quality of life?" 92,3 % (35/38) of the patients answered "good" or "very good" (vs. 72,0 % in the general population - p = 0.004).

Conclusion: Autologous fat grafting can be offered as a good alternative for total reconstruction after mastectomy with good aesthetic results and no deleterious impact on quality of life.
Title: Variations in breast reconstruction rate in France according to patient and site characteristics: A nationwide retrospective study of nearly 20,000 patients

Claudia Regis¹, Joconde Le⁴, Gwenael Le Teuff⁴, Malgorzata Cucchi¹, Loic Boulanger¹, Karine Hannebicque¹, Sylvia Giard¹, Marie-Pierre Chauvet¹, Julie Quemenr¹ and Marie-Cécile Ledeley². ¹Centre Oscar Lambret, Lille, France; ²Centre Oscar Lambret, Lille, France; ³Biostatistics Unit, Gustave Roussy, Villejuif, France and ⁴Centre Oscar Lambret, Lille, France.

Body: Background: Breast reconstruction (BR) for women who undergo mastectomy for cancer offers psychological benefits and improves quality of life. However, its use remains limited, especially for women over 65 years, with a large degree of international variation. The aim of this study was to find out factors influencing the surgical decision of BR in France where cancer related healthcare costs are fully reimbursed.

Methods: We used the French medico-administrative database to identify all primary mastectomies for breast carcinoma in 2012 and studied the rate of immediate (IR) or delayed breast reconstruction (DR) up to December 2015. Variations of BR rates were evaluated according to
- patient age, social deprivation index,
- profile of the hospital where the mastectomy was performed: type of hospital (cancer center, CC; university hospitals, UH; private, PrivH; or public, PubH), and hospital activity (surgical acts for breast cancer in 2012);
- disparities across administrative regions in terms of number of CC or UH, number of plastic surgeons, gynecologist-obstetrician surgeons and general surgeons in the region.

A hierarchical three-level logistic regression was used with SAS GLIMMIX to model the probability of BR taking into account clustering of observations (patients in hospitals, hospitals in regions). Splines were used to explore the functional form of the relationship between continuous variables and BR rate. Akaike information criterion was used for model selection.

Results: Among the 19,466 women who had a mastectomy in 2012, 5,328 (27.4%) subsequently had a BR: IR for 13.7% and DR for 13.7%. The BR rate significantly varied with age (p<0.0001), resulting in a much smaller BR rate in patients older than 65 compared to younger (7.5% vs 42.1%, p<0.0001). In case of BR, IR was more frequent than DR in older patients (66% of BR), whereas both were equally balanced before 65. BR rates decreased with increasing social deprivation index (from 32.7% to 21.5%, from the first to the fourth quartile of the distribution). BR rates significantly varied according to hospital type (35.0% in CC, 29.8% in UH, 25.9% in PrivH and 18.6% in PubH). BR rates were significantly lower in small activity hospital (varying from 13.4% in hospital with <=50 annual breast surgery to 35.1% in hospitals with >500), especially in older patients (varying from 3.1% to 10.3%). We also observed important heterogeneity of BR rates across administrative regions, but these variations were not explained by the number of CC or UH, the number of plastic surgeons, the number of gynecologist-obstetrician surgeons or the number of general surgeons in the region. In multivariate analysis, BR rate was significantly associated with age (p<0.0001), social deprivation index (p<0.0001), type of hospital (p=0.002) and hospital activity (p<0.0001), with persistent heterogeneity across administrative regions.

Conclusions: We identified substantial variations in BR rates across the French hospitals. Controlling for possible confounders, older patients have less breast reconstruction. This apparent heterogeneity can be part of women choice, however it suggests unequal access to high quality procedures for older women with breast cancer.
2017 San Antonio Breast Cancer Symposium

Publication Number: P4-13-04

Title: Robotic nipple-sparing mastectomy with immediate prosthetic breast reconstruction: A preliminary study

Benjamin Sarfati¹, Samuel Struk¹, Nicolas Leymarie¹, Jean François Honart¹, Françoise Rimareix¹, Suzette Delaloge¹, Mahasti Saghatchian¹, Amal Ghouadni¹, Héba Alkhashnam¹, Chafika Mazouni¹ and Frédéric Kolb¹. ¹Gustave Roussy, Villejuif, France.

Body: Background: Robotic nipple-sparing mastectomy (RNSM) could be a significant advancement in the treatment of selected breast cancers and prophylaxis. The aim of this prospective study was to assess feasibility of the RNSM with immediate prosthetic breast reconstruction (IPBR) on the first 50 consecutive cases performed in Gustave Roussy.

Methods: RNSM with IPBR was offered to patients with breast cup size A, B or C and ptosis grade ≤ 2. In case of oncologic surgery, RNSM was offered only if the patient fulfilled oncological indications for NSM (NAC). In case of prophylactic surgery, RNSM was offered only if a high-risk genetic mutation had been identified or if the patient was at high risk of breast cancer. The primary endpoint was the conversion rate to open technique. The duration of the surgery, the rate of postoperative complications, the length of the scar, the duration of drainage and hospital stay, the rate and duration of rehospitalization were also analyzed.

Results: Of 50 RNSM with IPBR from December 2015 to April 2017, 1 case of conversion to open technique occurred (2%) because of an uncontrolled bleeding from an internal mammary perforator. Two infections occurred (4%): one led to implant loss. There were no cases of mastectomy skin flap or NAC necrosis. No other major complications were observed. The duration of the whole procedure was reduced gradually from 214 min to 86 min. Length of scar was between 2.5 and 5 cm. Patients were discharged home between the 4th and the 10th postoperative day after the removal of the drain.

Conclusion: Preliminary data attest to the feasibility, the reproducibility and the safety of this approach. However, long-term data are needed to confirm the reduced rate of skin and NAC necrosis compared to the open technique, the oncological safety and the aesthetic stability of the result.
Title: Detection of local recurrence in premenopausal patients treated with neoadjuvant chemotherapy and mastectomy with or without breast reconstruction

Clare E Jacobson¹, Margaret Kozak¹, Emily Walck¹, Erin Hawley¹ and Kathleen Horst¹. ¹Stanford University School of Medicine, Stanford, CA.

Body: Background: Women who undergo mastectomy for breast cancer treatment often undergo implant based or autologous reconstruction. There are limited data, however, as to whether reconstruction may interfere with detection of a locoregional recurrence. The goal of this study was to assess whether women who undergo reconstruction after mastectomy have an increased risk of local recurrence and/or longer time to local recurrence detection.

Methods: One hundred and fifty-four premenopausal patients who underwent neoadjuvant chemotherapy followed by mastectomy were identified between 2005 and 2015. Patients with de novo Stage IV disease or insufficient medical records were excluded from analysis. Patient and treatment variables were collected, including clinical stage, type of chemotherapy, type of surgery and reconstruction, use of postmastectomy radiotherapy, and use of endocrine therapy. Local recurrence (LR) was defined as a chest wall or skin recurrence. Regional failure (RF) was defined as recurrence in the axilla, supraclavicular fossa, or internal mammary nodes.

Results: The median follow up for this cohort was 49.9 months. Of the 154 patients, 71 (46%) underwent unilateral mastectomy and 83 (54%) underwent bilateral mastectomies. Thirty patients (19%) elected to forgo reconstruction while 78 (51%) received tissue expander/implant based reconstruction, 29 (19%) received autologous reconstruction, and 17 (11%) had unknown reconstruction histories. Patients who had reconstruction had an increased time to detection of a LR compared to those without reconstruction (p=0.048). However, controlling for the T and N stage of disease, Cox regression demonstrated no observable difference in risk for LR between patients who underwent reconstruction compared to those who didn't. There was no difference in detection of RF between those with and without reconstruction (p=0.092).

Conclusions: Premenopausal patients treated with neoadjuvant chemotherapy followed by mastectomy with any type of reconstructive surgery had an increased time to local recurrence detection compared to those without reconstruction. There was no difference in the risk for local recurrence between those who underwent reconstruction compared to those who didn't.
Title: Does large volume displacement oncoplastic surgery still offer an advantage of a low positive margin rate using the new SSO/ASBrS/ASTRO margin guidelines?

Michael M Jonczyk¹, Krishnabhai Patel¹, Roger Graham¹, Abhihek Chatterjee¹, John Erban¹ and Lillian Chen¹. ¹Tufts Medical Center, Boston, Ma.

Body: Purpose
Breast conservation has become the mainstay of surgical management for early stage breast cancer. Large volume displacement oncoplastic surgery (LVOS) uses reconstructive mastopexy and breast reduction techniques to allow for larger oncologic resections while providing good aesthetic outcomes in a single operation. Oncoplastic surgery publications have recently increased by 220%, demonstrating the increasing popularity of this surgical technique,² and many of these studies have demonstrated excellent oncologic outcomes.⁴ To date, however, no study has used the most recent SSO/ASBrS/ASTRO surgical margin recommendations to assess oncoplastic surgery.⁴ Recent SSO/ASBrS/ASTRO guidelines established no ink on tumor as an adequate margin for invasive breast cancer and at least 2mm as adequate margins for ductal carcinoma in situ. The purpose of this study was to investigate the surgical margin rates of LVOS using the new SSO/ASBrS/ASTRO guidelines. We presumed that under the newer, stricter guidelines, LVOS would have a higher positive margin rate than reported in the past literature.

Methods:
Our study consisted of two parts. First, a literature review to assess margin rates before the introduction of SSO/ASBrS/ASTRO guidelines was done using PRISMA guidelines with an international Pubmed search and reviewed by two blinded authors. The search included keywords such as “oncoplastic breast surgery,” “lumpectomy,” “partial mastectomy,” and “positive margins associated with breast surgery.” All articles either pertained to LVOS, standard lumpectomy (SL) or both. The inclusion criteria for our study included histology discrepancy, and new guideline margin status. From this, we determined the published positive margin for SL and LVOS. Second, we analyzed all LVOS performed at our institution since the adoption of the new SSO/ASBrS/ASTRO margin guidelines and compared these margin rates to the literature review outcomes using Z tests.

Results:
Our study consisted of 1702 patients. There were 847 patients in LVOS group and 855 patients in the SL group. Of the 45 papers evaluated, 34 were not included due to exclusion criteria (missing: new margin guidelines, histology, or margin status). The pre-guideline positive margin rate for LVOS was lower than with SL (12.51% vs. 20.4%, P-value <0.001). Of the 50 LVOS operations done at our institution since adoption of the SSO/ASBrS/ASTRO margin guidelines, no statistical difference in the positive margin rates was noted when compared to the literature rates (10% vs. 12.67% respectively, P-value 0.5796). Positive margin rates for LVOS at our institution were lower than SL margin rates reported in the literature (P-value 0.0358).

Conclusions:
This study demonstrates that even with the stricter margin SSO/ASBrS/ASTRO guidelines, LVOS still has a low positive margin rate comparable to pre-guideline literature reports. LVOS continues to have a significantly lower positive margin rate than SL. This is the first study to report margin rates for LVOS after the adoption of the SSO/ASBrS/ASTRO guidelines, and confirms the importance of LVOS in providing optimal oncologic outcomes for patient with large locally advanced breast cancer.
Determinants in decision-making process of breast reconstruction in women over 65 years old

Julie Quemener¹, Jennifer Wallet¹, Loïc Boulanger¹, Karine Hannebicque¹, Sylvia Giard¹, Marie Pierre Chauvet¹ and Claudia Regis¹. ¹Centre Oscar Lambret, Lille, France.

Background: Breast cancer is the most common cancer among European women with 54,000 new cases in France in 2015. Nearly 47% of these cancers are diagnosed in women aged 65 and over. Mastectomy is still needed in 30% of cases, resulting in significant physical and psychological consequences. Breast reconstruction (BR) can reduce the effects of surgical treatment and improve quality of life. However, less than 20% of women choose BR in France. This number drops to 6% for patients over 65 years old. The objective of the study was to find the factors influencing the decision-making process for attempting breast reconstruction in women who are over 65.

Methods: We included retrospectively all patients over 65 years old who had an immediate or delayed breast reconstruction in our Cancer Center from January 2006 to July 2016. We set up a control group matching them with patients treated by mastectomy during the same period who did not choose BR. The matched-pair criteria were age, TNM stage and performans status, obtained from multidisciplinary consultation meeting database. We mailed to all patients a specially-designed questionnaire inspired by the BREAST-Q aimed at assessing the medical information that was delivered to them about BR and the reasons to choose or not choose reconstruction. The qualitative and quantitative results were analyzed. The two groups were compared using Chi-square, Fisher's exact, Mann-Whitney, and Student t test.

Results: Among 134 patients, 103 (77%) completed the questionnaire. Dedicated information on BR before the mastectomy was provided more frequently to patients who had BR (91.7% vs 66.7% p=0.008). Forty-one percent of patients sought out sources of information other than their surgeon (other physicians, friends, other patients, the internet – no significant differences between the two groups, p=0.1). The three most important persons influencing the decision-making process were first the patient's surgeon, second the patient's husband, and third her general practitioner (GP). These people were more often in favor of reconstruction in the BR group than in the mastectomy group (respectively, 94.5% vs 22.9% p<0.001; 65.4% vs 7.1 % p<0.001; and 64.2% vs 19.4%, p<0.001). Women judged that their age was an obstacle to reconstruction at the rate of 66.7% for the mastectomy group and at the rate of 3.8% in the BR group (p= 0.001). None of the women reported that her surgeon considered her age to be an obstacle for breast reconstruction. Women in the mastectomy group reported more fears about reconstruction than the women in the BR group (p< 0.001). Patients had less opportunity to talk about their fears with their surgeon in the mastectomy group (19.4% vs 66.1% p<0.001).

Conclusions: Providing dedicated information at the time of initial support is crucial in the choice of BR for women over 65. Patients' surgeons played a central role in the decision, but their GPs and husbands also provided important input. This dedicated information should help women over 65 to conclude that their age should not be a limiting factor for the decision to attempt breast reconstruction.
Title: Robotical-assisted laparoscopy for latissimus dorsi flap harvesting in breast reconstruction : A 23 consecutive cases report

Gilles Houvenaeghel¹, Sandrine Rua¹, Oona Franké¹, Monique Cohen¹ and Eric Lambaudie¹. ¹Institut Paoli Calmettes, Marseille, France.

Body: Background: Latissimus dorsi (LD) flap is a classic and usual procedure for immediate or secondary breast reconstruction with good results and poor complication rate. The principal issue is the long and often painful dorsal scar. The feasibility of laparoscopic LD flap harvesting and more recently robotically-assisted laparoscopy were described in short series. We report in this article the largest series of robotically-assisted laparoscopic LD flap harvesting. We describe its feasibility and the immediate and early post operative outcomes.

Material et methods: Between January 27th and December 21th of 2016, we performed 23 robotically-assisted laparoscopic LD flap harvestings in immediate and secondary breast reconstruction. Every patient was systematically sent for an evaluation of aesthetic result, pain and satisfaction on the second, the sixth and the twelth month.

Results: 78,3% of surgeries were realized for infiltrative breast cancer. 17 (73,9%) LD flaps were harvested in immediate reconstruction after nipple or skin sparing mastectomies (NSM -34,8%-or SSM -43,5%). The global mean operative time was 360,1 minutes, including bilateral and robotically-assisted mastectomies.

The mean hospital stay duration was 5 days (2-8 days).

We described one failure for secondary LD flap reconstruction (infection). The other cases resulted in successful reconstructions without heavy complications.

Discussion: Our series is the largest reported. It confirms the feasibility of a robotically-assisted procedure in breast reconstruction with LD flaps. Our study describes a reliable option for LD flap breast reconstruction without any additional scar. Some procedures were combined in selected patients with a robotic NSM with a single axillar incision.
Title: Immediate breast reconstruction versus delayed breast reconstruction: An analysis of oncological outcomes

Elizabeth S Morrow¹, Ross D Dolan¹, Viviane Blackhall² and Laszlo Romics². ¹Academic Unit of Surgery, Glasgow University, United Kingdom and ²New Victoria Infirmary, Glasgow, United Kingdom.

Body: Introduction
Breast reconstruction is an important option for patients who undergo mastectomy for breast cancer. Several studies have investigated outcomes for patients who undergo either immediate or delayed reconstruction versus mastectomy alone but few have evaluated the relationship of the timing of reconstruction to oncological outcome.

Aim
To determine if there is a difference in oncological outcomes for patients who undergo delayed versus immediate breast reconstruction following mastectomy for breast cancer.

Methods
Patients who underwent immediate or delayed breast reconstruction between 2005 and 2006 were identified from a database maintained prospectively at the regional plastic surgery unit. Tumour pathology details were obtained retrospectively from the electronic patient record and from local electronic laboratory systems. Details of treatment, and recurrence and mortality data were obtained by review of each patient’s electronic record. In the delayed reconstruction cohort, patients who underwent reconstruction 6-60 months after initial cancer surgery were included. In the immediate reconstruction group, patients who had recurrence or died within the first 6 months after surgery were excluded. Logistic regression survival analysis was carried out for the two cohorts and compared using Chi square test.

Results
193 patients who underwent immediate reconstruction and 116 patients who underwent delayed reconstruction were identified. Patients who had immediate reconstruction were more likely to have DCIS only, compared to those who had delayed reconstruction, but otherwise there was no significant difference between the two groups in terms of pathological characteristics or type of reconstruction performed (autologous or implant-based). Of those who had delayed reconstruction, median time from initial cancer surgery to reconstructive surgery was 27 months (6-58 months). There were 49 breast cancer deaths, 13 deaths from other causes and 65 recurrences. Median follow up time from reconstruction, of those who survived, was 111 months (29-134 months). Median follow up from initial cancer surgery was 116 months (46-185 months). There was no difference in breast cancer specific survival between the two groups when measured from time of cancer surgery (delayed reconstruction HR 1.05, 95% CI 0.59-1.89, p=0.861) or from time of reconstruction (delayed reconstruction HR 1.33, 95% CI 0.75-2.40, p=0.334). There was no difference in recurrence rates between the two groups when measured from time of cancer surgery (delayed reconstruction HR 0.94, 95% CI 0.56-1.60, p=0.822) or from time of reconstruction (delayed reconstruction HR 1.23, 95% CI 0.73-2.07, p=0.433).

Conclusion
Our data has demonstrated no difference in cancer specific survival or recurrence rates in patients who underwent mastectomy with immediate breast reconstruction compared to patients who had delayed reconstruction.
Title: Autologous immediate and delayed breast reconstruction utilizing micro fat grafts with and without dermatocutaneous flaps: A novel minimally invasive approach for reconstruction of small and medium size breasts

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Body: Background: Immediate breast reconstruction following mastectomy is a key quality metric of a comprehensive breast cancer program. Reconstructive options include autologous ( DIEP, TRAM, Latissimus) and implant/ADM reconstruction. DIEP, TRAM, and Latissimus flaps are invasive procedures that require prolonged operative times, extended recoveries, and have donor site morbidity (scars, weakness, hernia, etc). Implant/ADM reconstruction historically has not provided the same aesthetic satisfaction as autologous reconstruction, and also requires monitoring and ultimate replacement of the prosthetic device. Fat grafting has been found to be a safe and effective adjunct to standard breast reconstructive techniques. Fat graft only breast reconstruction has been reported, but in conjunction with external suction based tissue expansion. Dermatocutaneous flaps have been described for immediate breast reconstruction, but only in large breasted women (Goldilocks technique). We used micro fat grafts alone (no pre expansion) and in combination with dermatocutaneous flaps at mastectomy, to reconstruct small to medium sized breasts. We have applied the fat graft only technique to both immediate and delayed reconstructions.

Purpose: To present a novel, minimally invasive approach to reconstruction of small and medium sized breasts utilizing immediate or delayed micro fat grafts with and without immediate dermatocutaneous flaps. We also present immediate single stage bilateral breast reconstruction utilizing fat grafts, dermatocutaneous flaps, and nipple reconstruction. The authors will review patient selection criteria, surgical technique and present before and after photos.

Results: 12 non radiated breast cancer patients underwent immediate reconstruction of 21 breasts, utilizing fat grafting (1 breast) or dermatocutaneous flaps and fat grafting (20 breasts). An additional 5 patients underwent a total of 8 delayed fat graft only breast reconstructions. A total of 17 patients underwent reconstruction of 29 breasts. One patient underwent immediate reconstruction of one breast and delayed reconstruction of the other. Average age=54 years. Average BMI=27.4. All immediate breast reconstruction patients were discharged home next day. Patients underwent a mean=2 (range 1-5) fat graft sessions. Average fat injected per session was 153 ml (range 50-325). For immediate fat graft reconstructions, volume of fat grafted at the time of the mastectomy averaged 76 ml (range 55-100 ml). Total mean follow up from first procedure was 9 months, range 3-24 months.

Conclusion: The authors present a simple, minimally invasive approach to immediate and delayed breast reconstruction of small to medium sized breasts. Our approach is novel in that it combines fat grafts with dermatocutaneous flaps for immediate reconstruction and utilizes serial fat grafts without pre expansion for complete delayed breast reconstruction. The technique has been successfully utilized to complete both immediate unilateral and bilateral breast reconstruction in a single stage in select patients and with serial fat grafts in others. This is the largest series to date using this approach.
Title: A complication analysis between complete and partial tissue expander coverage using autologous flaps in cases of immediate breast reconstruction

Kazuyuki Kubo1, Atsumori Hamahata1, Katsunori Tozuka1, Miki Tsuboi1, Yuji Hayashi1, Ken Takai1, Takashi Saito1, Hiroyuki Sakurai2 and Hiroshi Matsumoto1. 1Saitama Cancer Center, 780 Komuro, Ina, Kita-Adachi, Saitama, Japan and 2Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku-ku, Tokyo, Japan.

Body: Background:
To avoid tissue expander exposure following mastectomy flap necrosis, several methods for covering expander by autologous flap in cases of immediate breast reconstruction have been reported. These methods are classified into two groups, complete or partial expander coverage. Two methods have potential risks of postoperative complications following: insufficient lower pole expansion and cranial migration in complete coverage methods, and lateral migration in partial coverage methods. However, the comparisons of complication rates between these two methods have not been reported. This study aims to compare the incidence of expander exposure following mastectomy flap necrosis and expander migration between two methods.

Methods:
A retrospective review of 93 patients (99 breasts) who underwent immediate expander-based breast reconstruction was performed. Patients were divided into two groups, complete or partial expander coverage by autologous flaps. In both groups, expanders were placed into subpectoral position. In partial coverage group, the lateral borders of pectoralis major muscles were sutured to the mastectomy skin flaps. If the skin flap was too thin to be sutured, the serratus anterior muscro-fascial flap was dissected and sutured to the lateral border of pectralis major muscle to cover the expander completely. Allograft products were not used in both groups. Demographics, intraoperative findings, and postoperative complications were compared between two groups.

Results:
Of the 99 breasts, 56 underwent complete expander coverage and 43 underwent partial coverage. Mastectomy flap necrosis rate was higher in the complete coverage group (Complete 14.3% versus Partial 0%; p=0.0091), however, there was no incidence of expander exposure in both groups. Lateral migration rate was higher in the partial coverage group (Complete 0% versus Partial 9.3%; p=0.033). There was no difference in cranial migration rate between two groups (Complete 12.5% versus Partial 2.3%; p=0.133).

Conclusions:
The thinness of the mastectomy flaps was considered to provide the higher incidence of mastectomy flap necrosis in the complete coverage group. The complete expander coverage reduced lateral migration rate and prevented expander exposure in cases of mastectomy flap necrosis.
**2017 San Antonio Breast Cancer Symposium**

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**Title:** Immediate one-stage implant-based breast reconstruction without an acellular dermal matrix in Japanese breast cancer patients

Seiko Okumura¹, Satsuki Tatibana¹, Chikayoshi Narita¹, Ikuo Hyodo¹, Masataka Sawaki², Masaya Hattori², Akiyo Yoshimura², Junko Ishiguro³, Haruru Kotani³, Naomi Gondo², Yayoi Adati², Hiroji Iwata² and Yuzuru Kamei³. ¹Aichi Cancer Center Hospital, Nagoya, Aichi, Japan; ²Aichi Cancer Center Hospital, Nagoya, Aichi, Japan and ³Nagoya University, Nagoya, Aichi, Japan.

**Body: Background.** In immediate one-stage implant-based breast reconstruction, acellular dermal matrix (ADM) has been usually used due to cover the outside part of the pectoralis major muscle in the world. Unfortunately, ADM is not approved in Japan yet. Therefore we have performed safety one-stage implant-based breast reconstruction without ADM according to several unique technics in our institution and obtain the excellent results. We report single institution experience using the retrospective medical data.

**Methods.** We chose the surgical method of immediate breast reconstruction (autologous and prosthetic) according to patient's preference, breast volume and cancer condition in early breast cancer patients (pts). Two hundred fifty four early breast cancer pts underwent immediate artificial breast reconstruction from January 2014 to March 2017 in our institution. Thirty seven pts were excluded in this analysis because of preplanned the expander insertion. ALLERGAN Natrelle 410 series were used as the silicon breast implant (SBI). We performed three unique steps to determine the one-stage SBI cases as follow. ICG fluorescence imaging to confirm the blood flow state of the chest skin after mastectomy is used as standard method. If there is a defective contrast region, we remove these area. And then, serratus anterior muscle/fascial and external oblique fascial flap is elevated to completely cover the SBI together with the pectoralis major muscle so that the between mastectomy field and the SBI insertion section are isolated. We used sizer to confirm the skin tension and form of breast. After confirming these steps, we adopted cases whether SBI insertion can be safety done.

We evaluated the total cosmetic finding based on three categories of inframammary fold position, balance of both breast size and form at half year after operation. If the difference between both inframammary fold positions was under 1cm, if compensatory pad is not necessary with underwear, if the protrusion of the lower pole is sufficient compared with the contralateral side, we gain the point one on each categories in each cases. The total cosmetic evaluation was classified as three categories. Good, fair and poor were defined as gained 3 points, 2 points and 0/1 points, respectively.

**Results.** Among 217 planned immediate prosthetic breast reconstruction pts, 186 pts (85.7%) were succeed one-stage implant-based breast reconstruction. Among 186 pts, the median age was 47 years old (23-75), median body weight was 53kg (35-84), median body mass index was 21.08 kg/m2(16.20-29.41), removed breast volume was 240g (56-522) and implant volume was 220cc (90-425). Implant removed in one case because of complications and infection was occurred in five cases. The revision surgery was performed in one cases because of malposition. Among all pts, the good, fair and poor of total cosmetic evaluation is 84.3%, 13.5% and 2.2%, respectively.

**Conclusions.** Immediate one-stage implant-based breast reconstruction without ADM may be useful and safe procedure according to our unique technics in early breast cancer patients who hope the immediate prosthetic breast reconstruction in Japan.
Title: The correlation between mastectomy specimen weight and volume- a guide to the choice of implant size in breast reconstruction

Body: Introduction: The concept of “conservative” mastectomy with breast reconstruction has led to a huge positive impact on the quality of life of breast cancer survivors. In 2015, 106,338 breast reconstructions were performed with implant-based reconstruction (IBR) constituting 86,013 (80.9%) of those procedures. Technically speaking, there is still no consensus on the most accurate method of assessing the size of implant used to achieve the desirable aesthetic results and symmetry. Some surgeons use the volume of the mastectomy specimen, believing that the volume of the implant replacing the volume of breast tissue removed is a logical way of thinking. Others prefer to depend on the weight of the specimen owing to the presumption that a mixture of fat and fibroglandular tissue will give an approximate overall density of 1.0g/cm3. To the authors' knowledge, the correlation between the mastectomy specimen volume and weight has been scarcely reported in the literature.

Materials and Methods: Patients undergoing nipple or skin-sparing mastectomy with immediate IBR at the London Breast Institute between January 2014 and December 2016 were included in this study. They were under the care of two senior Oncoplastic breast surgeons. Data on breast weight and volume as well as the size of implants used were prospectively collected. The volume of the breast tissue was assessed by volume displacement method while the weight was measured on a scale in grams. The exclusion criteria included patients with mastectomy specimen weighing more than 2000 grams. Further subgroups were divided into patients younger and those older than 50 years old. The presence or absence of cancer was also reviewed to assess whether the tumor tissue would have heavier weight when compared with volume.

Results: Between January 2014 and December 2016, a total of 236 mastectomies were performed, of which 144 were accompanied with IBR. The mean age of the patients was 45 years (range= 25-74). There were 79 right and 65 left breast specimens. Among these cases, 36 were bilateral. Tissue volume and weight had a strong direct correlation (N=144, R=0.99, P<0.00). Mastectomy volume had a marginally stronger correlation with implant volume/size (N=144, R=0.82, P=<0.00) than weight (N=144, R=0.79, P=<0.00). Further subgroup analysis showed that neither the presence of cancer nor the variation in breast density with age or menopausal status seemed to affect the correlation between the weight and volume of the breast tissue. 75% of reconstructions had implant size within 100 mls or grams of the mastectomy specimen.

Conclusions: Our study has shown that mastectomy specimen weight and volume have close enough correlation. The volume measurement was best estimated to the nearest 25 to 50 mls. On the other hand, the weight assessment was more accurate, objective, easier, and more reproducible with minimal inter-observer error. Hence, we believe that the breast weight can be reliably used to estimate the size of the implant. However, there are many other factors that should be taken into consideration when choosing an implant. For instance, the woman's wish for smaller or larger size, the width and height of the breast base, and the availability of a wide range of implants.
Title: Risk of recurrence and death in breast cancer patients after delayed deep inferior epigastric perforator flap reconstruction

Hannah Adam, Ann-Charlott Docherty Skogh, Åsa Edsander Nord, Inkeri Schultz, Jessica Gahm, Per Hall, Jan Frisell, Martin Halle and Jana de Boniface. 1 Karolinska Institutet, Stockholm, Sweden; 2 Karolinska University Hospital, Stockholm, Sweden; 3 Karolinska University Hospital, Stockholm, Sweden; 4 Karolinska Institutet, Stockholm, Stockholm, Sweden and 5 Capio St. Göran's Hospital, Stockholm, Stockholm, Sweden.

Body: PURPOSE
Post-mastectomy reconstruction using the deep inferior epigastric perforator (DIEP) flap is increasingly performed in breast cancer patients. The procedure induces large tissue trauma and it has been hypothesized that the release of growth factors, angiogenic agonists and immunomodulating factors may reactivate dormant micrometastasis. The aim of our study was to contrast the risk of breast cancer recurrence in patients undergoing DIEP reconstruction to patients treated with mastectomy alone.

PATIENTS AND METHODS
We conducted a retrospective nested case-control study. Cases were defined as breast cancer patients operated with delayed DIEP reconstruction at Karolinska University Hospital, Sweden, between 1999-2013. Three controls, defined as breast cancer patients operated with conventional mastectomy without delayed reconstruction, were matched to each case based on age, tumour stage and year of mastectomy. The primary endpoint was breast cancer-specific survival. Survival analysis was carried out by Kaplan–Meier survival estimates and Cox proportional hazard regression analysis.

RESULTS
In all, 254 cases and 729 controls were included and had a median follow up of 134 and 122 months, respectively (p=0.004). Breast cancer recurrence occurred in 50 (19.7%) cases and 174 (23.9%) controls, respectively (p=0.171). Ten-year breast cancer-specific survival was 90.7% for cases and 85.2% in controls (p=0.067). The corresponding figures for 10-year overall survival was 89.6% and 80.0%, respectively (p<0.001). Higher tumor stage and positive axillary lymph nodes, but not DIEP reconstruction, were independent risk factors for death due to breast cancer.

CONCLUSION
Our findings did not support the hypothesis that breast cancer patients undergoing DIEP reconstruction would have a higher rate of breast cancer recurrence than patients undergoing mastectomy alone.
A retrospective head-to-head comparison between TiLoop Bra/TiMesh and seragyn in 320 cases of reconstructive breast surgery

Christian Eichler¹, Carolin Schulz¹, Klaus Brunnert² and Warm Mathias¹,³. ¹Breast Center Cologne Holweide, Germany; ²Klinik für Senologie Osnabrück, Germany and ³Unifrauenklinik Koeln, Germany.

Body: Background: As an alternative option to ADMs (acellular dermal matrices) synthetic meshes are a cheaper and equally effective option in implanted breast reconstruction. However, clinical data concerning these synthetic meshes is limited. Also, direct comparisons between titanium-coated polypropylene mesh (TiLoop Bra/TiMesh) and partially absorbable polypropylene mesh (Seragyn) have not yet been reported. Therefore, this work will report clinical complication rate data on 320 cases of synthetic meshes TiLoop Bra/TiMesh and Seragyn.

Methods: This analysis represents a retrospective single surgeon multi-center study of 320 cases over 14 years (2003 until 2016) using either TiLoop Bra/TiMesh (n=192) or Seragyn (n=128) in breast reconstruction. Patient recruitment was consecutive. 124 cases of the TiLoop Bra /TiMesh cohort and 74 cases in the Seragyn cohort were oncological interventions. Results were correlated with ADM based reconstructions (Epiflex and SurgiMend) performed by the same surgeon.

Results: Major complication rates (i.e. revision surgery) occurred in 3.9 % (Seragyn) and 8.3 % (TiLoop Bra/TiMesh) of all cases. (not statistically significant). Minor complications (i.e. seroma requiring aspiration, infection requiring antibiotics, red breast syndrome/rash (RBS) and wound dehiscence occurred in 18 % (Seragyn) and 8.9 % (TiLoop Bra/TiMesh). Overall minor complication rates did also not differ statistically significant. However, subgroup analysis showed RBS to occur more often in the Seragyn group (3.9 % Seragyn vs. 0.5 % TiLoop Bra/TiMesh, p<0.05). A significant difference between SurgiMend and Epiflex compared to the synthetic meshes could also not been shown.

Conclusion: This retrospective analysis shows that titanium-coated polypropylene meshes like TiLoop Bra/TiMesh and partially absorbable meshes like Seragyn do not differ significantly in complication rates. Compared to the exponentially more expensive ADM alternatives complication rates did also not differ significantly. Synthetic meshes seem to be a cheaper and equally effective option.
Impact of immediate breast reconstruction after mastectomy on the outcome of patients receiving neoadjuvant chemotherapy

Hiroko Nogi, Shoichi Tomita, Makiko Kamio, Hisashi Shioya, Yasuo Toriumi and Hiroshi Takeyama. Jikei University School of Medicine, Minato-ku, Tokyo, Japan.

Body:

Background and purpose
In breast cancer patients receiving neoadjuvant chemotherapy (NAC), immediate breast reconstruction (IBR) is controversial. IBR might favor recurrences and metastases due to delayed adjuvant radiation therapy. We retrospectively investigated whether IBR after mastectomy influenced the outcome of patients receiving neoadjuvant chemotherapy.

Patients and methods
Between 2006 and 2016, 243 breast cancer patients received total mastectomy after NAC, 48 of whom underwent IBR. Patients receiving IBR (IBR group) were compared to patients who did not receive IBR (no-IBR group) over a prolonged median follow-up time (72.3 months).

The regimen was 4 cycles of epirubicin (100 or 75 mg/m2), 5-fluorouracil (500 mg/m2), and cyclophosphamide (500 mg/m2) followed by 4 cycles of docetaxel (75 mg/m2). Post-mastectomy radiation was applied in cases treated by IBR following the same selection criteria as for standard mastectomy regardless of the reconstruction approach.

Results
Patients in the IBR group were on average younger than patients in no-IBR group (p<0.001). The percentage of patients with clinical T1/2 tumor was 81.2% in the IBR group and 58.4% in no-IBR group (p=0.0042). 2 patients (4.2%) in the IBR group and 9 patients (4.6%) in no-IBR group showed the locoregional recurrences. 2 patients (4.2%) in the IBR group and 20 patients (10.3%) showed distant metastases. There were no significant differences.

Conclusions
IBR after total mastectomy was not associated with worse rate of locoregional recurrences in patients receiving NAC.
Title: Eclectic breast reconstruction: A tailored approach

Alejandro Maciel-Miranda¹, Luz M Gutiérrez-Zacarías¹ and Juan E Bargalló-Rocha¹. ¹Instituto Nacional de Cancerología, Mexico City, Mexico.

Body: Methods that are eclectic combine whatever seems the best or most useful things from many different areas or systems, rather than following a single system.

There are several publications that compare one single technique against another: expander versus flap, pedicled flap versus free flap, etc. At Instituto Nacional de Cancerología (INCan) we are able to offer several techniques for breast reconstruction: tissue expander/implant, direct-to implant, TRAM flap, DIEP flap, Latissiumus Dorsi flap, and the use of dermal acellular matrix.

In an eclectic approach we propose to use any of these techniques, depending on patient characteristics, individual risk estimation and shared decision-making approach.

We performed 110 breast reconstructions applying this innovative approach.

Patient demographic and clinical characteristics (n=110)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) mean ± standard deviation (SD)</td>
<td>43 (±9.7)</td>
</tr>
<tr>
<td>20-40 years</td>
<td>41 (37.3%)</td>
</tr>
<tr>
<td>40-50 years</td>
<td>40 (36.4%)</td>
</tr>
<tr>
<td>&gt;50 years</td>
<td>29 (26.4%)</td>
</tr>
<tr>
<td>Smoking (n %)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>7 (6.4%)</td>
</tr>
<tr>
<td>No</td>
<td>84 (76.4%)</td>
</tr>
<tr>
<td>Pasive</td>
<td>1 (.9%)</td>
</tr>
<tr>
<td>Discontinued</td>
<td>18 (16.4%)</td>
</tr>
<tr>
<td>BMI mean ± standard deviation (SD)</td>
<td>26.35 (±4.05)</td>
</tr>
<tr>
<td>Normal weight</td>
<td>45 (40.9%)</td>
</tr>
<tr>
<td>Overweight</td>
<td>40 (36.4%)</td>
</tr>
<tr>
<td>Obesity</td>
<td>25 (22.7%)</td>
</tr>
<tr>
<td>Breast volume (cc) median (p25-p75)</td>
<td>425 (350-500)</td>
</tr>
<tr>
<td>Unilateral</td>
<td>88 (80%)</td>
</tr>
<tr>
<td>Bilateral</td>
<td>22 (20%)</td>
</tr>
<tr>
<td>Breast Cancer Stage n (%)</td>
<td></td>
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<tr>
<td>In situ</td>
<td>2 (1.8%)</td>
</tr>
<tr>
<td>IA</td>
<td>23 (20.9%)</td>
</tr>
<tr>
<td>IB</td>
<td>2 (1.8%)</td>
</tr>
<tr>
<td>IIA</td>
<td>28 (25.5%)</td>
</tr>
<tr>
<td>IIB</td>
<td>20 (18.2%)</td>
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<tr>
<td>IIIA</td>
<td>22 (20 %)</td>
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<tr>
<td>IIIB</td>
<td>4 (3.6%)</td>
</tr>
<tr>
<td>IIIC</td>
<td>4 (3.6%)</td>
</tr>
<tr>
<td>IV</td>
<td>1 (.9%)</td>
</tr>
</tbody>
</table>
Without breast cancer and BRCA mutation 4 (3.6%)  
Radiotherapy n(%)  
None 52 (47.3%)  
Prior to mastectomy 4 (3.6%)  
After mastectomy 54 (49.1%)  

We also implemented enhanced recovery after surgery protocols for same day surgery (in expander to implant exchange), and did quality of life and satisfaction evaluation with BREAST-Q questionnaires. Reconstruction surgeries are summarized in table 2.

Reconstruction characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reconstruction n(%)</td>
<td></td>
</tr>
<tr>
<td>Bilateral</td>
<td>22 (20%)</td>
</tr>
<tr>
<td>Unilateral</td>
<td>88 (80%)</td>
</tr>
<tr>
<td>Type of Reconstruction n(%)</td>
<td></td>
</tr>
<tr>
<td>Expander-implant</td>
<td>60 (54.5%)</td>
</tr>
<tr>
<td>Direct to implant</td>
<td>17 (15.5%)</td>
</tr>
<tr>
<td>Becker implant</td>
<td>3 (2.7%)</td>
</tr>
<tr>
<td>DIEP flap</td>
<td>15 (13.6%)</td>
</tr>
<tr>
<td>Oncoplastic</td>
<td>2 (1.8%)</td>
</tr>
<tr>
<td>Lattisimus dorsi flap</td>
<td>6 (5.5%)</td>
</tr>
<tr>
<td>TRAM flap</td>
<td>5 (4.5%)</td>
</tr>
</tbody>
</table>

Complications were: none in 55(50%), mastectomy flap necrosis 8(7.3%), infection 3 (2.7%), lost of reconstruction 4 (3.6%), capsular contracture 27 (24.5%).

BREAST-Q questionnaires showed 100% would recommend reconstructive surgery. 91.7% felt included in the decision process. Satisfaction with breast (preoperative vs postoperative) 66.21 vs 81.13, Satisfaction with outcome 85.06, Psychosocial well being 80.75 vs 88.35, Physical well-being 74.92 vs 68.73, Sexual well-being 66.66 vs 72.84, Satisfaction with information 86.86, Satisfaction with surgeon 98.73, Satisfaction with medical staff 96.2.

There are several options with advantages and disadvantages, treatment should be individualized based on risk estimation and shared decision making, then applying ERAS protocols, and evaluation with BREAST-Q questionnaires. Plastic surgeons should dominate several options, to fulfill patient expectatives, and high complexity options should be derived to high volume centers. Breast reconstruction technique should also be tailored to regional circumstances on economic and human resources, technical capacity; even factors like OR time, costs, infrastructure and health system.
Title: Changes in treatment and improved survival of inflammatory breast cancer (IBC/T4d) and T4a-c lesions: 1990-2014

Judith A Malmgren¹, Mary K Atwood² and Henry G Kaplan². ¹HealthStat Consulting Inc, Seattle, WA and ²Swedish Cancer Institute, Seattle, WA.

Body: Background: T4 breast cancer (BC) is a tumor of any size with direct extension to the chest wall and/or to the skin. Tumor stage T4a-d including IBC (T4d) are aggressive cancers, often presenting with distant metastases or progressing quickly to metastatic breast cancer (MBC).

Methods: We conducted a retrospective cohort analysis of invasive BC cases with T4 tumor stage from a prospectively collected institutional BC registry, years 1990-2014 [total N = 10,414, all T4 = 320 (T4a = 20, T4b = 130, T4c = 15, T4d (IBC) = 155)]. Presentation at diagnosis by hormone receptor (HR) and her2-neu (HER2) status, treatment, follow up for distant metastases and vital status were reviewed. Change in treatment over time was compared using Pearson chi square tests. Disease specific survival (DSS) was estimated using Kaplan Meier plots. Cox proportional hazards modeling was used to obtain T4 adjusted hazard ratio (HzR) among MBC (de novo and distant recurrence) patients (n=1158).

Results: T4 tumor stage cases were 3% of the total cohort but 10% of relapsed MBC and 28% of de novo MBC cases (p<.001). T4 cases were significantly more often HR negative (35%) (p < .001). After 1999 when HER2 results were available, T4 was significantly less often HR+/her2- (52%), and significantly more often HR+ or -/HER2+ (28%) and triple negative (20%) (TN = HR-/HER2-) than the rest of the cohort (p < .001). No change over time in number or percent treated with surgery, radiation, chemotherapy, neoadjuvant therapy, hormone therapy or combinations was observed. Chemotherapy significantly changed over time with the introduction of trastuzumab treatment for HER2+ disease in 1999 and increasing from 60% for HER2+ disease in 1990-2014 to 100% in 2005-14 (p < .001). The shift to combination doxorubicin/cytoxan/taxane from 13% in 1990-98 to 43% in 1999-2014 was also significant (p < .001). T4 5 year DSS improved from 45% in 1990-98 to 68% in 1999-2014 after the introduction of her2-neu testing and new treatments (p = .001). Survival improvement over time was significantly greater among T4 cases than T1-T3 cases [T1 = 2%, T2 = 5%, T3 = 5%, T4 = 23% (p < .001)]. In a Cox proportional hazards model of MBC patients, outcome = BC mortality, adjusted for race, diagnosis year, age, HR status, number and type of metastatic sites, T4 status was associated with an 18% decreased chance of mortality [HR=.82, 95% confidence interval =.68, .988, p =.048].

Cox proportional hazards model (n=1158)

<table>
<thead>
<tr>
<th>factor</th>
<th>HzR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR-</td>
<td>1.64 (1.41, 1.91)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>age 70+ years</td>
<td>1.97 (1.65, 2.34)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>recurrent MBC vs de novo MBC</td>
<td>1.91 (1.61, 2.26)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>&gt;= 2 metastatic sites</td>
<td>1.38 (1.19, 1.60)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>visceral dominant mets site</td>
<td>1.23 (1.05, 1.44)</td>
<td>.012</td>
</tr>
<tr>
<td>T4 primary tumor</td>
<td>.82 (.68, .998)</td>
<td>.048</td>
</tr>
</tbody>
</table>

Conclusions: T4 BC is disproportionately triple negative, HER2+, diagnosed as stage IV MBC or distant recurrent MBC compared to T1-T3 stage category disease. T4 disease, including IBC, has had the largest improvement in disease free survival over time of all BC subtypes and is a factor associated with better MBC survival. Despite the small overall number of patients presenting with T4 tumor stage disease, the dramatic reduction in T4 mortality over time has a large impact on survival improvement.
2017 San Antonio Breast Cancer Symposium

Publication Number: P4-14-02

Title: CSF1/CSF1R axis reprograms epithelial-to-mesenchymal phenotypes in inflammatory breast cancer

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¹The University of Texas MD Anderson Cancer Center, Houston, TX and ²Okayama University University Hospital, Okayama City, Okayama, Japan.

Body: Inflammatory breast cancer (IBC) accounts for 2% of breast cancers but 10% of breast cancer-related deaths in the US. Clinical hallmarks of IBC are tumor cell emboli in lymphatic vessels and overexpression of E-cadherin, which promotes cell clustering. Given these hallmarks, IBC is thought to spread via collective invasion and cell clusters. However, we showed that IBC cells underwent epithelial-to-mesenchymal transition (EMT) and metastasized through EMT. Thus, there are two contradictory theories of IBC metastasis. The objectives of this study were 1) to propose a model that reconciles these two models, and 2) to identify target molecules for inhibition of IBC metastasis. Methods: We previously showed that Matrigel culture induced EMT-like changes in SUM149 IBC cells. To test if this transformation from epithelial (E) to mesenchymal (M) in Matrigel culture is unique to IBC cells, a panel of breast cancer cells was cultured in both monolayer and Matrigel-coated plates. The cells were IBC (SUM149, SUM190, KPL4, IBC3), triple-negative breast cancer (TNBC) (MDA-MB-231, MDA-MB-468), and ER+ (MCF7) cells. Phenotypic changes in morphology and expression of EMT markers (E-cadherin, vimentin) were captured with bright field and immunofluorescent (IF) images, respectively. For genome-wide and targeted transcriptional analysis, SUM149 cells cultured in monolayer and Matrigel were processed using DNA microarrays and Taqman qRT-PCR. To correlate the Matrigel gene signature with M features in human breast cancer, a human breast cancer data set was hierarchically clustered with the Matrigel gene signature. Results: SUM149 cells showed a remarkable phenotypic change from E in monolayer culture to M in Matrigel. IF analysis confirmed induction of vimentin expression in Matrigel but stable expression of E-cadherin (thus, we refer to this state as E/M hybrid). This trend was also observed with SUM190 cells. Using qRT-PCR, we confirmed downregulation of E-cadherin and upregulation of M markers (vimentin, Twist1, Snail1, ZEB2) in Matrigel-cultured SUM149 and SUM190 cells compared to monolayer-cultured cells. DNA microarray transcriptional analysis confirmed this trend in SUM149 cells. TNBC has more M-like features than other breast cancer subtypes. Given this evidence, we clustered human breast cancer data using overexpressed genes in Matrigel-cultured SUM149 cells. We identified a cluster of 20 genes in TNBC samples and, assuming that these genes are drivers of E to M transition, chose the inflammation-related gene CSF1 as a candidate. The CSF1/CSF1R axis was inhibited by a CSF1R inhibitor, BLZ945; moreover, treatment with BLZ945 reversed the EMT changes in cells in Matrigel culture. Treatment with 5 µM BLZ945 re-induced E-cadherin expression and suppressed Snail1 and Twist1 expression in Matrigel-cultured SUM149 cells. Conclusion: IBC cells are more prone to undergo transition from E to E/M phenotype in Matrigel culture than are cells of other breast cancer subtypes. The CSF1/CSF1R axis plays a role in this E to E/M transition, thus warranting testing its significance using an in vivo IBC model. Phenotypic transition and reversion between E and E/M phenotypes could be a new paradigm that reconciles two contradictory models of IBC metastasis.
Title: Integration of clinical and pathological data with the DCIS score to predict the risk of local recurrence

Lawerence Paszat¹, Rinku Sutradhar¹, Limei Zhou², Nafisha Lalani³, Sharon Nofech-Mozes¹ and Eileen Rakovitch¹. ¹University of Toronto, Toronto, ON, Canada; ²Institute for Clinical Evaluative Sciences, Toronto, ON, Canada and ³Sunnybrook Health Sciences Centre, Toronto, ON, Canada.

Body: Background: Prediction of local recurrence (LR) risk after breast-conserving surgery (BCS) for ductal carcinoma in situ (DCIS) is needed to guide decisions regarding risks and benefits of adjuvant radiotherapy (RT). We aim to determine the optimal combination of clinical and pathological characteristics with the Oncotype DCIS Score (DS) to predict individualized 10 year risks of local recurrence (LR) after BCS (with or without RT) for DCIS and develop a web-based nomogram / risk calculator.

Methods: DS (continuous, categorical risk groups low/intermediate/high) and complete clinico-pathological data (age, tumor size, nuclear grade, comedonecrosis, multifocality, margin width and receipt of breast RT) are available for 1102 cases from the Ontario population cohort of pure DCIS treated by BCS (981 cases with negative margins, 121 cases with positive margins). We examined various categorizations of discrete variables, and transformations of continuous variables, and used model selection procedures to determine the best fitting Cox proportional hazards regression model of LR according to the c-statistic, Akaike Information Criterion, and log-likelihood. We tested all two-way interactions and interactions with time. The 10-year probability of LR was calculated for each woman using the estimate of the baseline survival function and the estimate of the linear predictor, which is a function of the regression parameter estimates and specific covariate values. Model calibration will be explored by comparing observed versus predicted risk of LR, and the model's discriminative ability will be assessed by the concordance index. Model validation will be conducted via bootstrapping approaches.

Results: In the best fitting main effects full model, the adjusted hazard ratios (HR) (95% confidence intervals (CI)) for LR included: intermediate/ high risk DS vs. low risk (HR =1.96 (1.39, 2.74)), age < 50 years at diagnosis vs. age>= 50 (HR = 1.62 (1.16, 2.25)), square root of tumor size (HR/mm = 1.24 (1.11, 1.38)), comedonecrosis > 30% vs. <=30% (HR=1.53 (1.08, 2.16)), multifocality (HR=2.01 (1.45, 2.77)), and receipt of RT (HR=0.50 (0.37, 0.68)). There was a significant interaction between tumor size and DS but not between DS and RT. Among women with a low risk DS and age >= 50, tumor size <= 10 mm, <= 30% comedo necrosis, no multifocality, low or moderate nuclear grade and negative margins, the average predicted 10 year LR risk = 6.8% (range 6.4% - 7.6%) after treatment by BCS without RT, and 3.6% (range 3.4% - 3.8%) after BCS+RT (an absolute benefit of 3.2% from RT). Among women with intermediate/high risk DS and the same low risk clinical-pathological features, the average predicted 10 year LR risk = 19.0% (range 18.3% - 20.0%) without RT, and 9.5% (range 9.1% - 10.3%) with RT (an absolute benefit of 9.5% from RT).

Conclusion: This prediction model combines clinical and pathological features with the DS to improve estimates of local recurrence risk after BCS alone and the absolute benefit with RT, which can improve decision making in DCIS. After calibration and validation, it will be the basis of a web-based nomogram / risk calculator. It also demonstrates the importance of molecular testing for studies of the de-escalation of therapy for DCIS.
Impact of radiotherapy and endocrine therapy on further events: Final multivariate analysis of a prospective, national cohort study of screen detected ductal carcinoma in situ (DCIS) of the breast

Alastair M Thompson¹, Karen Clements², Shan Cheung², Sarah E Pinder³, G Lawrence³, Elinor Sawyer³, Olive Kearins², Graham R Ball⁴, Ian Tomlinson⁵, Andrew M Hanby⁶, Jeremy Thomas⁷, Anthony J Maxwell⁸, Matthew G Wallis⁹ and David J Dodwell¹⁰. ¹The University of Texas MD Anderson Cancer Center, Houston, TX; ²Public Health England; ³Guy's Hospital; ⁴Nottingham Trent University, Nottingham, United Kingdom; ⁵Oxford NIHR Comprehensive Biomedical Research Centre, Oxford, United Kingdom; ⁶St James Hospital, Leeds, United Kingdom; ⁷Western General Hospital, Edinburgh, United Kingdom; ⁸University of Manchester, Manchester, United Kingdom; ⁹Cambridge University Hospitals, Cambridge, United Kingdom and ¹⁰University of Oxford, Oxford, United Kingdom.

Body: Key words: DCIS, radiotherapy, endocrine therapy, survival, surgical margins

Background:
The benefits and risks of breast screening remain controversial, with particular concern that ductal carcinoma in situ (DCIS) may be over-diagnosed and over-treated. There is little prospective data on treatment or outcomes for screen detected DCIS.

Methods:
A prospective cohort of non-invasive lesions diagnosed through the United Kingdom National Health Service Breast Screening Programme (NHSBSP) (1 April 2003 to 31 March 2012) was linked to national databases and case note review to analyse patterns of care, recurrence and mortality.

Results:
Screen-detected DCIS in 9938 women was analysed, 33% (9938/30041) of women with a final diagnosis of non-invasive breast neoplasia diagnosed through the NHSBSP over the same time.

The patients (mean age was 60 years: range 46-87 years) were treated by breast conservation surgery (BCS; 7007; 70.5%) or mastectomy (2931). At 64 months median follow up, 697 (6.8%) had further DCIS or invasive breast cancer after BCS (7.8%) or mastectomy (4.5%) (p<0.001) and 228 women (2.3%) developed contralateral malignancy.

Breast radiotherapy (RT) after BCS (4363/7007; 62%) was associated with a 3.1% absolute reduction in any ipsilateral DCIS or invasive cancer (No RT: 7.2% vs RT: 4.1% (p<0.001) and a 1.9% absolute reduction for ipsilateral invasive breast recurrence (No RT: 3.8% vs RT: 1.9% (p<0.001), independent of excision margin width or size of DCIS. Women who did not receive RT after BCS had more ipsilateral events (p=0.008) when the radial excision margin was <2mm. RT was rarely used after mastectomy for DCIS (33 women). Adjuvant endocrine therapy (prescribed for 1208/9938; 12.2%) was associated with a reduction in any ipsilateral recurrence, independent of whether women did (HR 0.57: 95% CI 0.41 - 0.80) or did not (HR 0.68: 95% CI 0.51 - 0.91) receive RT after BCS.

Among 321 (3.2%) women who died, 46 deaths (0.5%; 14.3% of all deaths) were attributed to invasive breast cancer. Death from breast cancer was uncommon and outnumbered 5:1 by death due to other causes. RT after BCS was associated with a non-significant 0.2% absolute reduction in breast cancer mortality. However, women who developed invasive breast cancer had a worse survival than those with further DCIS (p<0.001).

Conclusions:
Recurrent DCIS or invasive cancer is uncommon following screen detected DCIS treated by surgery and adjuvant therapy. Both RT and endocrine therapy following surgery were associated with a significant reduction in further DCIS and invasive disease, but not breast cancer mortality, within 5 years of diagnosis. This study quantifies the benefits of radiotherapy and endocrine therapy to inform decision making in the management of screen detected DCIS.
**Title:** Tumor-infiltrating lymphocytes in breast ductal carcinoma in situ: Correlations with tumor pathobiology in a French cohort of 495 cases (BONBIS)

Beryl Bayol¹, Lucie Tixier-Deves², Marie Dauplat³, Fabrice Kwiatkowski⁴, Anne Cayre⁵, Catherine Abrial⁶, David Azria⁷, Frederique Penault-Llorca⁸ and Nina Radosevic-Robin⁹. ¹Centre Jean Perrin, Clermont-Ferrand, France; ²University Clermont Auvergne, INSERM U1240 "Molecular Imaging & Theranostic Strategies", Centre Jean Perrin, Clermont-Ferrand, France; ³Institute Paoli-Calmettes, Marseilles, France; ⁴University Clermont Auvergne, INSERM U1240 "Molecular Imaging & Theranostic Strategies", Centre Jean Perrin, Clermont-Ferrand, France; ⁵Equal Contribution University of Montpellier, INSERM U1194, Institute for Cancer Research of Montpellier, Montpellier, France and ⁶Equal Contribution University Clermont Auvergne, INSERM U1240 "Molecular Imaging & Theranostic Strategies", Clermont-Ferrand, France.

**Body:** **Background:** Numerous studies have shown important impact of tumor-infiltrating lymphocytes (TILs) on natural or therapeutically-modified evolution of invasive breast cancer (IBC), however knowledge about TIL role in breast ductal carcinoma in situ (DCIS) is still limited. Because of the lack of reliable prognostic parameters, DCIS treatment is much less personalized than IBC therapy. BONBIS is a phase 3 French multicenter randomized trial designed to compare 2 schemes of adjuvant radiotherapy (adjRT) for DCIS (Azria et al, ASCO meeting 2011, TPS 131). It is accompanied by a translational study of DCIS pathobiology, aimed to discover predictive or prognostic biomarkers. Here we present results of TIL density (TIL-d) assessment, its correlation with pathobiology of the lesions and preliminary clues for further biomarker search in this DCIS cohort.

**Methods:** Formalin-fixed, paraffin-embedded DCIS surgical specimens, obtained before adjRT, were prospectively collected and centrally reviewed for histology (architectural pattern, nuclear grade, proliferation, presence of necrosis), receptor status (ER, PR, HER2) and TIL-d. TIL-d was assessed on H&E-stained DCIS sections and reported as percentage of the DCIS specialized stroma area occupied by lymphocytes, lympho-plasmocytes and plasmocytes. Tumors were classified using the St Gallen 2011 criteria for IBC (PMID 21709140). For purpose of this study, the HER2+ category included all cases with HER2 protein expression scored 2+ and 3+, irrespective of the ERBB2 amplification status. **Results:** TIL-d was assessed in 495 cases, with distribution as follows: 0-4% TILs (D1): 85.5% (n=423); 5-9% TILs (D2): 9.3% (n=46); ≥10% TILs (D3): 5.2% (n=26). Molecular subclasses of those cases were: luminal A (LumA): 39% (n=192); luminal B (LumB): 25.5% (n=126), HER2+: 28.5% (n=141) and triple-negative (TN): 7% (n=33). TIL-d significantly correlated with molecular subclass: ≥5% TILs (D2) were found in 39.4% (13/33) TN, 22.7% (32/141) HER2+, 18.2% (23/126) LumB and only in 1% (2/192) LumA cases (p<10⁻⁷). TIL-d of ≥10% (D3) was found only in 26/495 cases (5.2%) and of ≥50% only in one. Similarly to D2, D3 was most frequent in TN, and then in HER2+, LumB and LumA lesions (15%, 9.2%, 7.1%, 0.5%, resp., p<10⁻⁷). TIL-d significantly correlated with 2 architectural patterns: positively with solid (38.8%, 52.2% and 60% of D1, D2 and D3 within the solid lesions, resp., p=0.03) but negatively with cribriform (53%, 32.6% and 28% of D1, D2 and D3 within the cribriform lesions, resp., p=0.0027). Finally, the presence of necrosis was significantly associated with TIL-d of ≥5% (65.7% vs 84.5%, D1 vs D2+D3, p=0.071). Due to still short follow-up, the analysis of predictive factors for survival or adjRT benefit was not performed. **Conclusion:** This DCIS cohort is characterized by low density of TILs. Richer infiltration by TILs (≥10%) was found, as reported in IBC, in TN and HER2+ lesions, however ≥50% TILs was an extremely rare finding. Interestingly, the LumB cases had TIL-d comparable to the HER2+ cases. The associations between ≥10% TILs and molecular subclass or with DCIS architecture will be evaluated in the future as biomarkers of adjRT impact on survival in this cohort.
2017 San Antonio Breast Cancer Symposium

Publication Number: P4-15-04

Title: A longitudinal cohort study to identify risk factors for the development of invasive cancer in unresected DCIS

Anthony J Maxwell¹, Karen Clements², Bridget Hilton³, David J Dodwell³, Andrew Evans⁴, Kearnis Olive⁵, Sarah E Pinder⁶, Jeremy Thomas⁶, Wallis G Matthew⁷ and Alastair M Thompson⁸. ¹University Hospital of South Manchester, Manchester, United Kingdom; ²Public Health England, Birmingham, United Kingdom; ³University of Oxford, Oxford, United Kingdom; ⁴Ninewells Hospital and Medical School, Dundee, United Kingdom; ⁵Guy's Hospital, London, United Kingdom; ⁶Western General Hospital, Edinburgh, United Kingdom; ⁷Cambridge University Hospitals NHS Foundation Trust, Cambridge & NIHR Cambridge Biomedical Research Centre, Cambridge, United Kingdom and ⁸University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Background: The variable natural history of ductal carcinoma in situ (DCIS) remains poorly understood. Randomized trials of active surveillance versus guideline concordant care are currently underway: the Comparison of Operative to Monitoring and Endocrine Therapy (COMET) trial in the US, LOW Risk dcIS (LORIS) trial in the UK and Low Risk Dcis (LORD) in Europe. Given this context, we examined the outcomes of a contemporary group of women with DCIS who did not undergo initial surgical resection.

Methods: A longitudinal cohort of women diagnosed with DCIS on needle biopsy who did not undergo initial surgical excision for ≥1 year were identified through the Cancer Registry with case note and death certificate review for subsequent outcomes. Results: Eighty-nine eligible women with DCIS alone diagnosed on needle biopsy (most with 14-gauge core needle biopsy) between 1998 and 2010 were identified. The mean age at diagnosis was 72 years (range 44-94 years) with mean follow-up (diagnosis to death, invasive disease or last review) of 62 months (range 12-180 months). Twenty-nine women (33%) developed histologically proven invasive breast cancer, 28 at the site of the initial DCIS biopsy, after a mean interval of 54 months (range 12-144 months): 14/29 (48%) women originally had high grade DCIS, 10/31 (32%) intermediate grade and 3/17 (18%) low grade DCIS (initial grade not known in 12). Time to detect a diagnosis of invasive breast cancer was associated with initial grade of DCIS (p=0.0016, log-rank test): after mean intervals of 41 months (high grade), 69 months (intermediate grade) and 78 months (low grade) respectively. Younger age was associated with development of invasive disease (p<0.003, Mann-Whitney U-Test). High grade (grade 3) invasive breast cancer exclusively occurred in women with a prior diagnosis of high grade DCIS. Invasion was more frequent in lesions with calcification as the predominant feature than those without (23/50 v. 5/25; p<0.05, Fisher exact test). Forty-four women were prescribed endocrine therapy, use of which was associated with a lower rate of invasive breast cancer (p=0.05). Ultimately 18 women underwent surgery, 17 for invasive cancer. The mean interval from DCIS diagnosis to death was 76 months for those who developed invasive cancer; 48/89 women died, 12 had a certified cause of death as breast cancer.

Conclusion: High grade DCIS, mammographic microcalcification and lack of endocrine therapy were associated with progression to invasion. The findings suggest surgical excision of high grade DCIS should continue but provides support that women with DCIS features which include low grade should be considered for the COMET, LORIS or LORD active surveillance trials.
Title: The presence of one or multiple foci of microinvasion is not associated with an increased risk of local recurrence in women with ductal carcinoma in situ treated with breast conserving therapy

Nafisha Lalani1,2,3, Lawrence Paszat1,2,3, Rinku Sutradhar2,3, Sumei Gu2, Cindy Fong2, Sharon Nofech-Mozes4, Wedad Hanna4, Alan Tuck5, Bruce Youngson6, Naomi Miller6, Susan J Done6, Martin C Chang11, Sandip Sengupta7, Leela Elavathil8, Prashant A Jani9, Michel Bonin10 and Eileen Rakovitch1,2,3. 1University of Toronto, Toronto, ON, Canada; 2Institute for Clinical Evaluative Sciences, Toronto, ON, Canada; 3Sunnybrook Health Sciences Centre, University of Toronto, Toronto, ON, Canada; 4Sunnybrook Health Sciences Centre, University of Toronto, Toronto, ON, Canada; 5London Health Sciences Centre, London, ON, Canada; 6University Health Network, Toronto, ON, Canada; 7Kingston General Hospital, Kingston, ON, Canada; 8Juravinski Cancer Centre, Hamilton, ON, Canada; 9Thunder Bay Regional Health Sciences Centre, Thunder Bay, ON, Canada; 10Sudbury Regional Hospital, Sudbury, ON, Canada and 11Mount Sinai Hospital, Toronto, ON, Canada.

Body: Background: Ductal Carcinoma in Situ (DCIS) is a non-invasive breast cancer often treated with breast-conserving surgery (BCS) with or without radiotherapy (RT). It is unclear if the presence of microinvasion (MI) (invasion ≤1mm) is associated with an increased risk of LR (DCIS or invasive) or invasive LR compared to women with pure DCIS. In addition, the impact of multiple foci (>2) of MI compared to pure DCIS is also unknown; therefore, it is unclear if some women with MI require more aggressive treatment. We evaluated the impact of the presence of MI and the number of foci of MI on the risks of any LR and invasive LR in a population of women with DCIS with and without MI treated with BCS.

Methods: The cohort includes all women diagnosed with pure DCIS or DCIS with MI in Ontario from 1994-2003 treated with BCS +/- RT. All cases had systematic pathology review to confirm the presence and number of foci of MI. Treatment and outcomes were ascertained through administrative databases and validated by chart review. Cox proportional hazards model was used to evaluate the impact of MI and the number of foci of MI (1 vs >2 foci) on the development of any LR and invasive LR compared to cases with pure DCIS. The 10-yr local recurrence-free survival (LRFS) and invasive LRFS rates were calculated using the Kaplan-Meier approach with differences compared using the log-rank test.

Results: The population cohort includes 2,988 women with DCIS treated by BCS (N=2,721 pure DCIS, N= 267 DCIS with MI). Median follow-up (12 years; p=.23) and median age at diagnosis (58 years; p=.17) were similar in both groups. RT was given in 58% of cases with MI and 51% of cases with pure DCIS (p=.03). Hormonal therapy was utilized in 7.1% of women with MI and 5.3% of women with pure DCIS (p=.22). LR developed in 59 (22.1%) cases with MI and 530 (19.6%) cases of pure DCIS. Women with MI were more likely to have high nuclear grade (p<.001), and larger tumor size (p<.001) compared to those without MI. On multivariable analyses adjusted for age, the presence of 1 focus of MI (HR=.92, 95% CI:.64-1.33) or ≥2 foci of MI (HR=1.26, 95% CI:.85-1.85) was not associated with an increased risk of any LR compared to cases with pure DCIS. Factors associated with any LR were age <50 years at diagnosis, RT, multifocality and high nuclear grade. The presence of 1 focus of MI (HR=.86, 95% CI:.52-1.40) or ≥ 2 foci of MI (HR=1.45, 95% CI:.90-2.32) was also not associated with an increased risk of invasive LR compared to cases of pure DCIS. Among women treated with BCS alone, the 10 year LRFS rates were 80%, 75% and 73% for women with pure DCIS, 1 focus, ≥2 foci of MI (p=.10). The invasive LRFS rates were 89%, 91% and 85% (p=.26). Among women treated with BCS+RT, the 10 year LRFS rates were 87%, 88% and 80% (p=0.32) for women with pure DCIS, 1 focus or ≥2 foci of MI. The invasive LRFS rates were 93%, 90% and 86% (p=.44). There was no interaction between the presence of MI and RT.

Conclusions: Women with DCIS with one or multiple foci of microinvasion (≤1mm) treated by breast conserving therapy do not have an increased risk of LR or invasive LR compared to women with pure DCIS.
Title: Low dose tamoxifen lowers recurrences after mastectomy for in situ neoplasia. Ten-year results of a monoinstitutional study

Aliana Guerrieri-Gonzaga1, Sara Gandini1, Davide Serrano1, Matteo Lazzeroni1, Giancarlo Pruneri1, Clara Varricchio1, Massimiliano Cazzaniga1, Maria Cristina Leonardi1, Viviana Galimberti1, Giuseppe Viale1, Andrea De Censi2 and Bernardo Bonanni1. 1European Institute of Oncology, Milan, Italy and 2E.O. Ospedali Galliera, Genoa, Italy.

Body: There is no agreement upon the need of a preventive treatment after breast mastectomy for in situ neoplasia. Low-dose tamoxifen (5 mg/day) has comparable antiproliferative effect than the standard dose of 20 mg/day in biomarker trials and has been shown to halve ipsilateral recurrence in a large cohort of postmenopausal ER positive DCIS treated with breast conserving surgery (Guerrieri-Gonzaga et al., Int J Cancer 2016).

Here we investigated the effect of low dose tamoxifen in patients treated with mastectomy for an in situ neoplasia and followed-up in a single Institution for a median of 10 years.

Our cohort consists of 404 consecutive premenopausal (n=281) or postmenopausal (n=123) women who underwent unilateral mastectomy at the European Institute of Oncology (IEO), with or without nipple preservation, between 1996 and 2011. Patients had a diagnosis of pure LCIS (n=12) or ER positive (ER>1%) DCIS (n=363) or both (n=29) and were treated with tamoxifen 5 mg/day (n=162) or no treatment (n=242) upon medical judgment, patient preference and/or clinical trial assignment. The main subject and tumor characteristics are reported in table 1.

Patient and tumor characteristics

<table>
<thead>
<tr>
<th></th>
<th>No tam (n=242)</th>
<th>Low dose tam (n=162)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age, years (IQR)</td>
<td>46 (41, 54)</td>
<td>47 (42, 51)</td>
<td>0.65</td>
</tr>
<tr>
<td>Premenopausal status (n, %)</td>
<td>160 (66)</td>
<td>121 (75)</td>
<td>0.07</td>
</tr>
<tr>
<td>Median BMI (kg/m2, IQR)</td>
<td>22 (20, 25)</td>
<td>22 (20, 24)</td>
<td>0.7</td>
</tr>
<tr>
<td>Breast cancer family history (%)</td>
<td>29</td>
<td>32</td>
<td>0.44</td>
</tr>
<tr>
<td>Histology (LCIS, DCIS, both; %)</td>
<td>4/92/4</td>
<td>2/86/12</td>
<td>0.01</td>
</tr>
<tr>
<td>Grading (G1,G2,G3;%)</td>
<td>11/59/29</td>
<td>18/61/20</td>
<td>0.04</td>
</tr>
<tr>
<td>Median ER (% , IQR)</td>
<td>90 (70, 95)</td>
<td>90 (80, 95)</td>
<td>0.005</td>
</tr>
<tr>
<td>Median PgR (% , IQR)</td>
<td>40 (5, 80)</td>
<td>68 (25, 90)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Median Ki67 (% , IQR)</td>
<td>15 (10, 23)</td>
<td>14 (10, 20)</td>
<td>0.03</td>
</tr>
<tr>
<td>Radiotherapy (n, %)</td>
<td>95 (39)</td>
<td>76 (47)</td>
<td>0.13</td>
</tr>
</tbody>
</table>

After a median follow-up of 10 years (range 4-21 years) and a median low dose tamoxifen treatment of 4.9 years (IQR 2.7, 5.0), a total of 85 events were observed (28 in situ, 40 invasive breast cancers, 3 metastatic diseases, 12 other primary cancers, 2 deaths). A total of 36 ipsilateral breast events (23 versus 13 in the no tam versus tam group, respectively), 32 contralateral breast events (22 versus 10 in the no tam versus tam, respectively) and 17 other events (11 versus 6 in the no tam versus tam, respectively) occurred. Overall, eleven deaths (3%) occurred and no endometrial cancers were observed. A time-dependent competing risk model was applied for tamoxifen use and we have shown that low-dose tamoxifen was associated with a 48% reduction on all breast events (adjusted HR=0.52, 95% CI: 0.31–0.88, p=0.01), adjusting for radiotherapy and age.

Although limited by the observational nature of the study, we show for the first time that treatment with low dose tamoxifen is effective and safe in women who underwent mastectomy for non-invasive breast neoplasms and should be taken into consideration as a risk reduction strategy for premenopausal and postmenopausal women with breast intraepithelial neoplasia.
Title: Quantifying the natural history and overtreatment rate of ductal carcinoma in situ

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Body: Background. Subsets of ductal carcinoma in situ (DCIS) are thought to be relatively indolent disease with long progression time to invasive cancer. Nevertheless, the vast majority of women diagnosed with DCIS undergo extirpative surgery, potentially leading to widespread overtreatment of patients who would not develop symptomatic breast cancer in absence of treatment. Due to a poor understanding of the natural history of untreated DCIS, it remains difficult to derive estimates of the true rate of overtreatment. The objective of this study was to quantify the natural history of untreated DCIS through synthesis of retrospective data sources and to estimate the associated rate of overtreatment.

Methods. A systematic PubMed search was performed to identify published studies on ipsilateral invasive cancer-free survival in women who did not undergo surgery with curative intent after diagnosis with DCIS. Individual life histories from included studies were manually extracted and aggregated in a patient-level meta-analysis. Each lesion was assigned to one of three categories: DCIS precursor lesions, low-risk DCIS, and high-risk DCIS. Time-to-event analyses (Kaplan-Meier) and Cox proportional-hazards models were used to calculate absolute and relative progression rates for the three risk groups. For the low-risk group, overtreatment rates were estimated by means of a competing risk analysis. For this purpose, progression hazards were estimated by maximum likelihood inference of parametric mixture models, and non-breast cancer mortality rates were derived from standardized life tables (birth cohort: 1960).

Results. A total of n=122 women from 3 retrospective studies were included in the patient-level meta-analysis. The median age at diagnosis was 47 years (interquartile range [IQR]: 41-57) and median follow-up was 17 years (IQR: 6-20). Compared to DCIS precursor lesions (n=38), relative rates of progression to invasive cancer were significantly higher for both low-risk DCIS (n=68; hazard ratio [HR]: 4.8, 95%-CI: 1.4-16.0) and high-risk DCIS (n=16; HR: 6.9, 95%-CI: 1.7-27.8). Among women with low-risk DCIS, the cumulative progression to ipsilateral invasive disease after 5, 10, and 20 years was found to be 16.9% (95%-CI: 9.7-28.5), 28.5% (95%-CI: 16.6-38.8) and 35.1% (95%-CI: 23.8-49.8), respectively. The corresponding overtreatment rates for low-risk DCIS were estimated to be of the order of 56%, 63% and 72% for ages at diagnosis of 55, 65 and 75 years respectively.

Conclusion. To our knowledge, this study constitutes the most comprehensive quantitative analysis of the natural history of incompletely treated DCIS. The estimated propensity to progress to invasive disease suggests overtreatment among some women diagnosed with low-risk DCIS, especially in older patients with comorbidities. The extent to which this cohort resembles those currently diagnosed with DCIS is unclear. However, these results underscore the importance of clinical trials in evaluating active surveillance and/or anti-estrogen therapy as alternative management strategies for women diagnosed with low-risk DCIS.
**2017 San Antonio Breast Cancer Symposium**

**Publication Number:** P4-15-08

**Title:** Association of OncotypeDX® DCIS Score™ results with local recurrence in patients with DCIS treated on accelerated partial breast radiotherapy (APBI) protocols

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**Body:**

**Background:** Ductal carcinoma in situ (DCIS) is a proliferation of malignant epithelial cells of the ducts and terminal lobular units of the breast that do not invade the basement membrane. The incidence of DCIS has increased markedly since the early 1980s, chiefly due to screening mammography. Whole breast radiotherapy has largely been used to treat breast DCIS after lumpectomy. More recently, APBI has increasingly been utilized for breast DCIS. Currently updated American Society of Radiation Oncology (ASTRO) APBI guidelines have included "low risk" DCIS (as defined by RTOG 9804 criteria). The following results further explore clinico-pathologic factors, in addition to the DCIS Score, in order to better define an appropriate DCIS population for APBI.

**Methods:** An exploratory analysis aimed to retrospectively measure the association between clinico-pathologic factors and the DCIS Score result, an optimized 12-gene expression algorithm, and risk of any local failure (in situ or IBC recurrence) in a cohort of women treated with local excision and APBI on prospective phase II (NCT01185145) and phase III (NCT01185132) clinical trials. Multifocal tumors were described only by local pathology and not determined or defined centrally. The DCIS Score assay was performed by quantitative RT-PCR on formalin-fixed paraffin-embedded DCIS tumor specimens by Genomic Health (Redwood City, CA). Descriptive statistics of the cohort and assay results overall and by clinical trial were derived. Univariable Cox proportional hazards regression was used to determine whether there was an association between local failure and categorized DCIS Score group (≥39 vs <39) or other clinico-pathologic factors on the pooled cohort of clinical trial patients.

**Results:** This analysis included 104 evaluable patients (N=18 from NCT01185145 and N=86 from NCT01185132). The median age was 60 (range: 41-80), 79% of patients were postmenopausal, and the median span of DCIS was 6 mm (range 2-25 mm). Over two-thirds of the cohort presented with necrosis (71%). The distribution of DCIS Score results ranged from 0 to 82, with 69% of patients having a DCIS Score result <39. The median follow-up time was longer at 8.2 years in NCT01185145 versus 3.0 years in NCT01185132. There was a total of 6 local recurrences. DCIS Score result was significantly associated with local recurrence in univariable modeling (hazard ratio=10.3 for ≥39 vs <39; p=0.0104). None of the other clinico-pathologic characteristics resulted in any significant correlation with locoregional recurrence. All results were highly variable due to the small number of events.

**Conclusion:** The DCIS Score assay demonstrated risk stratification in this cohort of patients treated with local excision and APBI pooled from two clinical trials. These results are consistent with those recently published by Rakovitch et al (*J Natl Cancer Inst* 2017). The cohort in this study was dominated by those in the phase III trial. Due to the small number of local recurrence events and limited follow-up time in the phase III trial, caution should be taken when interpreting the results. Further investigations are needed to confirm findings.
Title: Refined estimates of local recurrence risk in a clinical utility study: Integrating the DCIS score, patient age and DCIS tumor size

Jennifer B Manders¹, Lawrence J Solin², Charles E Leonard³, Eleftherios P Mamounas⁴, Ruixiao Lu⁵, Michelle Turner⁶, Frederick L Baehner⁵,⁶ and Julia White⁷. ¹The Christ Hospital, Cincinnati, OH; ²Albert Einstein Health Network, Philadelphia, PA; ³Rocky Mountain Cancer Centers, Denver, CO; ⁴UF Health Cancer Center at Orlando Health, Orlando, FL; ⁵Genomic Health, Inc., Redwood City, CA; ⁶University of California, San Francisco, San Francisco, CA and ⁷Duke University Medical Center, Durham, NC.

Body: Background: Better tools are needed to estimate the risk of local recurrence (LR; DCIS or invasive) after breast-conserving surgery (BCS) for pts with DCIS in order to inform treatment decisions. Traditional clinico-pathologic (CP) factors, e.g., age and tumor size, provide an average LR risk derived from clinical trials and population studies. The Oncotype DX 12-gene DCIS Score assay has been validated to provide individual 10 yr LR risk estimates (Solin JNCI 2013; Rakovitch BCRT 2015). Previously we reported the impact of the DCIS Score result on radiotherapy (RT) recommendations including the pre-assay LR risk and RT recommendation and the change in RT recommendation from pre- to post-assay (Manders Ann Surg Oncol 2016). Recently a patient specific meta-analysis (MA) combined data from E5194 and Ontario DCIS Cohort (ODC) adjusting for pertinent clinico-pathologic factors to provide refined prediction estimates of LR risk after BCS alone (Rakovitch ASCO 2017). Herein we applied these risk estimates integrating DS, tumor size and patient age with adjustment for diagnosis in the year 2000 or later to refine estimates of LR in DCIS patients from the Manders et al study.

Methods: 13 U.S. sites enrolled pts with DCIS treated with BCS alone from 3/2014 to 5/2015. Pts with LCIS but no DCIS, invasive BC, or planned mastectomy were excluded. Data were prospectively collected on CP factors, physician estimates of LR risk, and DCIS Score. Refined estimates of 10-yr risk of LR are presented by DCIS Score result category (0-38; 39-54; 55-100), age group (≥50 vs <50 yr) and tumor size (≤1; >1-2.5; >2.5 cm).

Results: Of the 127 pts enrolled, median age was 60 yr,79.5% were postmenopausal. Median size was 8mm & 39% were ≤5mm. Median margin width was 3.0mm. ER and PR by IHC were positive in 89% and 78% of pts, respectively. For patients ≥50 yr with tumors ≤1 cm and low risk DS, the 10-yr LR risk ranges from 5.3-10.0%. A high DS result is associated with a higher 10-yr median predicted risk of LR in all subsets (table 1). The DCIS Score integrated with tumor size and patient age and the adjustment for diagnosis in 2000 or later provided risk estimates that are often lower than those provided by the DCIS Score alone without adjustment for diagnostic year. Using DS alone the percentage of patients with risk of LR <8% was 0%; however, incorporating patient age and tumor size with the DS and adjusting for diagnosis in 2000 or later, it increased to 30.9% of patients.

Conclusions: Integration of the DCIS Score assay, that provides individual risk estimates of LR, with patient age and DCIS tumor size and adjusting for diagnosis in 2000 or later, provides refined estimates of 10-yr LR risk after BCS alone for DCIS. This integration enhances prognostic LR risk estimates and frequently provides lower risk estimates with which to guide individualized treatment decisions.

Distribution of 10-year risk of local recurrence using DCIS Score (DS), tumor size, and age, adjusting for diagnosis in 2000 or later.

<table>
<thead>
<tr>
<th>Tumor Size(cm)</th>
<th>Low DS (&lt;39)</th>
<th>Inter DS (39-54)</th>
<th>High DS (≥55)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(Yr)</td>
<td>N</td>
<td>Median (Min-Max)%</td>
<td>N</td>
</tr>
<tr>
<td>≤1</td>
<td>≥50</td>
<td>45</td>
<td>7.0 (5.3-10)</td>
</tr>
<tr>
<td>&lt;50</td>
<td>8</td>
<td>10.3 (7.4-12.1)</td>
<td>4</td>
</tr>
<tr>
<td>&gt;1-2.5</td>
<td>≥50</td>
<td>24</td>
<td>9.5 (7.3-12.6)</td>
</tr>
<tr>
<td>&lt; 50</td>
<td>2</td>
<td>16.4 (16.1-16.7)</td>
<td>2</td>
</tr>
<tr>
<td>&gt;2.5</td>
<td>≥50</td>
<td>5</td>
<td>15.7 (14.9-23.7)</td>
</tr>
<tr>
<td>------</td>
<td>-----</td>
<td>---</td>
<td>-----------------</td>
</tr>
<tr>
<td>&lt;50</td>
<td>0</td>
<td></td>
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</tbody>
</table>
Title: Second breast events after DCIS: Where, what and when?

Lauren M King, Min Yi, Savitri Krishnamurthy, Kelly K Hunt and Alastair M Thompson. 1The University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Background: Detection and diagnosis of ductal carcinoma in situ has substantially increased since the widespread use of mammographic screening with the incidence of DCIS increasing between 1975 from 5.8 per 100,000 to 33.8 per 100,000 women in 2010. While surgical resection of DCIS remains the main treatment, outcomes in terms of in breast re-occurrence or contralateral disease are unpredictable. Recently, three large clinical trials of active surveillance versus standard of care have been initiated. We sought to compare the clinical, imaging, histopathological features and treatment of DCIS for contemporary patients who subsequently developed a second in situ or invasive breast lesion among women treated within a single institution.

Methods: A contemporary prospective cohort study comprising 2,509 women treated for DCIS between 2000 and 2014 was examined for diagnostic imaging, surgery, pathology, adjuvant treatment and second breast events. Patients with primary bilateral DCIS or a bilateral second event were excluded as were patients with antecedent invasive breast cancer. Patients were grouped into two populations based on ipsilateral and contralateral second breast event.

Results: Amongst 2,509 women treated for DCIS between 2000 and 2014, of whom 146 had a second breast event (5.8%). 77 (52.7% of events, 3.07% overall) developed an ipsilateral second breast event (37 DCIS, 40 invasive breast cancer) and 69 (47.3%, 2.75%) patients developed a contralateral second breast lesion (34 DCIS, 35 invasive breast cancer). Patients who developed a contralateral second event were older than those who developed an ipsilateral second event (58 years vs. 52 years, p=0.003). Patients receiving segmental mastectomies were more likely to develop an ipsilateral second event than a contralateral event (85.7% vs. 60.9%, p=0.001). Patients who developed a second contralateral breast event were significantly older at diagnosis (63 years vs. 58 years, p=0.003). There was no significant difference in the number of (69 vs. 77) or time between contralateral and ipsilateral second breast events (5.2 years and 5.1 years, p=0.8) nor in the number of patients with invasive or in situ second events (75 vs. 71). Patients with ipsilateral second breasts events were more likely to undergo bilateral mastectomy than those with contralateral second events (15 vs. 2, p=0.003).

Conclusions: Following standard of care treatment for DCIS, 1 in 20 women develop further DCIS or invasive disease at a median of 5 years with very similar proportions of ipsilateral or contralateral and DCIS or invasive disease. Current trials of active surveillance should consider a median 5 years follow up as a critical time point for reporting results.

Key Words: Ductal carcinoma in situ, invasive breast cancer, population-based cohort, surgery, radiotherapy, endocrine therapy.
Title: “Is it cancer or not?” A qualitative exploration of patient perspectives surrounding the diagnosis and treatment of DCIS

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Body: Background: Approximately 53,000 women in the US undergo treatment for ductal carcinoma in situ (DCIS) each year, of whom only 20-30% may benefit. To better understand both the clinical and psychosocial impact of a DCIS diagnosis and different management approaches, we sought to engage women with DCIS regarding their experience surrounding diagnosis (dx) and treatment.

Methods: In July 2014, we administered a web-based survey through an email listserv to the Susan Love Army of Women that resulted in over 2000 respondents self-identified as patients with DCIS. The survey included open-ended questions designed to assess patients’ perspectives on their experiences with the dx and treatment of their DCIS. Responses were coded using an inductive coding schema; common themes were identified and summarized. Women who reported an invasive cancer, a second primary or recurrent tumor, or other benign breast lesions (in absence of DCIS) were excluded from analysis.

Results: Among 1,857 women included in the analytic sample, the average age at dx was 60 years; 18% women were ≤2 years from their dx; most women (93%) identified as white. Four primary themes were identified: 1) uncertainty about DCIS dx; 2) uncertainty surrounding treatment; 3) concern about side effects from treatment; and 4) concern about recurrence and invasive breast cancer. Uncertainty about treatment often manifested as women questioning whether they were over-treated for their DCIS, “over-reacting by having surgery,” or wondering if “watchful waiting might be better.” In addition to recalling bothersome side effects and sequelae from both their local and systemic (hormonal) therapy, women also expressed doubt about their treatment choices, specifically, that they were not necessarily “doing enough” with many women citing recurrence, the “cancer spreading”, or becoming invasive, as primary concerns. Uncertainty about whether DCIS was cancer or not, was noted by many women, with one calling it a “grey zone” and others articulating that DCIS is “having a dx that's not really cancer... yet you might still lose your breast,” and experiencing “confusion about my status as a cancer patient - as in I wasn't sure if I even was a cancer patient. I had no idea where I fit in…”

Conclusion: A DCIS dx can be confusing and distressing, with women making treatment decisions based on a limited understanding of the disease, its risks, and pros and cons of treatment options. There is a need to develop additional strategies to improve the management of this disease and other screen-detected conditions, through better understanding of the disease and its outcomes, coupled with improved methods to communicate this information to those affected. Our study highlights the potential value of collecting patient reported outcomes (PROs) to inform clinical research and care. Ongoing clinical trials like the COMET, LORIS, and LORD studies, which incorporate robust PROs, should provide additional evidence for patients, health care providers and other stakeholders regarding the medical and psychosocial benefits and harms of different DCIS management options.
Evaluating the significance of the Van Nuys prognostic index for the management of ductal carcinoma in situ – one center’s experience

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**Body:** **Aim:** This was to assess the treatment outcomes of ductal carcinoma of the breast (DCIS) based on the Van Nuys Prognostic Index.

**Material and Methods:** 634 consecutive patients with DCIS were treated at the Warsaw Cancer Center, Poland between 1996 and 2008 based on the VNPI score. Tumorectomy without radiotherapy (T) was performed in 123 (20%) patients, breast conserving treatment (BCT) in 206 (32%) and mastectomy (M) in 305 (48%). Disease-free survival (DFS) and overall survival (OS) were analysed within 3 groups and a multivariate analysis was performed to determine the most important factor affecting local relapse.

**Results:** 5-year, 10-year and 15-year DFS in the M group were respectively 98%, 97% and 97% whilst 5-year, 10-year and 15-year DFS in the BCT group were respectively 91%, 87% and 80%. 5-year, 10-year and 15-year DFS in the T group were respectively 92%, 73% and 67%. Local recurrence was observed in 54 patients. In 40% cases this was noninvasive and in 60% invasive. Only 4 of 54 patients with recurrence died due to breast cancer. 5-year, 10-year and 15-year OS in the M group were respectively 96%, 92% and 89%. 5-year, 10-year and 15-year OS in the BCT group were respectively 99%, 94% and 85%. 5-year, 10-year and 15-year OS in the T group were respectively 95%, 90% and 73% (p=0.422). Tumor size assessed by mammography was the only variable affecting DFS.

**Conclusions:** Treatment outcomes of DCIS based on the Van Nuys Prognostic Index are comparable with those cited the literature, however, the recurrence rate in the T group seems to be very high. This group requires that new risk factors be sought for prior to taking a treatment decision.
**2017 San Antonio Breast Cancer Symposium**

**Publication Number:** P4-15-13

**Title:** When is cancer not really cancer? The PREvent ductal carcinoma in situ invasive overtreatment now (PRECISION)* initiative

Jelle Wesseling¹, Alastair Thompson², Serena Nik-Zainal³, Andrew Futreal², Shelley Hwang⁴, Jos Jonkers¹, Esther Lips¹, Daniel Rea⁵ and On Behalf of the PRECISION Team¹. ¹Netherlands Cancer Institute; ²MD Anderson Cancer Center; ³Welcome Trust Sanger Institute; ⁴Duke University and ⁵University of Birmingham.

**Body: Background**

Ductal carcinoma in situ (DCIS) now represents 20-25% of all breast neoplasia due to large-scale detection by widely adopted population-based breast cancer screening programs. As a result, thousands of women are confronted with DCIS each year: more than 8,000 in the UK, 2,500 in the Netherlands, and some 50,000 in the US. Conventional management includes surgery, supplemented by radiotherapy and/or endocrine therapy, but overtreats the majority of DCIS as ~1% recur annually and breast cancer mortality is ~3% at 20 years. Uncertainty as to which DCIS lesions will progress to invasive cancer or, after excision, which will return with recurrent DCIS or invasive breast cancer drives this overtreatment. This urges us to learn how to distinguish DCIS that may progress to invasive breast cancer from the majority of indolent DCIS. Such distinction may be best achieved by synergistic international collaboration between leading global experts from various disciplines, driven by the essential input from patient voices as full members of the research team.

**Aim**

PRECISION (PREvent ductal Carcinoma In Situ Invasive Overtreatment Now) aims to save thousands of women with low risk DCIS the burden of intensive inappropriate treatment of DCIS (surgery, radiation therapy, hormonal therapies) through the discovery of new data and development of novel tests that promote informed and shared decision-making between patients and clinicians, without compromising the excellent outcomes for DCIS management presently achieved.

**Methods**

First, three large DCIS cohorts and supplementary resources will be collected enabling in depth molecular studies. Second, extensive genomic characterization, immune profiling and imaging analysis will be performed. In vivo and in vitro modeling will be performed to study the biology of DCIS in detail. Finally, all clinical, immune, and molecular data will be incorporated into a clinical risk prediction model. This risk prediction model will be validated in three prospective randomized DCIS trials in the US (COMET trial), UK (LORIS trial), and mainland Europe (LORD trial).

**How the results of this research will be used**

The discoveries from our laboratory studies, including a risk stratification model, will be cross-validated in three prospective trials of DCIS active surveillance versus conventional treatment (the COMET, LORIS and LORD trials). As such, the main result of this study will be that we can identify a group of women for which active surveillance for DCIS could be a safer alternative to intensive treatment. Ultimately, this may also contribute to a more reassuring perception of risk regarding non-life threatening precancerous lesions in general, reducing anxiety and preserving quality of life.

* The PRECISION Team is a Cancer Research UK Grand Challenge Award 2017 winning team and will be jointly funded by Cancer Research UK and the Dutch Cancer Society.
Title: Personalized neoadjuvant treatment planning using optical metabolic imaging

Joe T Sharick1,2, Alex J Walsh2, Melinda E Sanders3, Mark C Kelley4, Ingrid M Meszoely4, Mary A Hooks4, Mark E Burkard5, Karla Esbona5, Alka Choudhary5 and Melissa C Skala1,6. 1Morgridge Institute for Research, Madison, WI; 2Vanderbilt University, Nashville, TN; 3Microbiology, and Immunology, Vanderbilt University, Nashville, TN; 4Vanderbilt University, Nashville, TN; 5University of Wisconsin, Madison, Madison, WI and 6University of Wisconsin, Madison, Madison, WI.

Body: Currently, there are no reliable methods to optimize treatment regimens for individual breast cancer patients. Oncologists choose drug treatments based on expression levels of tumor cell signaling receptors (i.e. HER2, ER, PR) and other factors, and assess whether the treatment is effective after significant time has passed. Unfortunately, over one third of patients exhibit resistance to their initial treatment, increasing their risk of future metastasis and death. Morbidities from sub-optimal drug regimens could be reduced with a personalized drug screen for breast cancer at the time of diagnosis. With the vast number of therapeutic options available to patients (>50 drugs approved with more on the way), a high-throughput screening technology is needed to accurately evaluate how a patient will respond to these options.

Here we present Optical Metabolic Imaging (OMI) of tumor-derived organoids as a predictive drug screening platform for individual breast cancer patients. Changes in cell metabolism precede changes in tumor volume and thus present an earlier marker of treatment response. OMI is sensitive to these early changes by exploiting the intrinsic fluorescent properties of NAD(P)H and FAD, coenzymes of metabolic reactions. OMI endpoints include the optical redox ratio (the fluorescence intensity of NAD(P)H divided by the fluorescence intensity of FAD), as well as the fluorescence lifetimes of NAD(P)H and FAD. The redox ratio reflects the cellular redox balance, and the fluorescence lifetimes report on the binding activity of these coenzymes. OMI has the unique ability to non-invasively monitor metabolism in living, intact samples on the single-cell level, and can thus quantify heterogeneity in drug response. Changes were quantified at the single-cell level using the OMI Index, a linear combination of the optical redox ratio and the mean NAD(P)H and FAD fluorescence lifetimes. This index was derived using a multivariate analysis of variance and has been shown previously to correlate with treatment response in human cancer cells. OMI also allows for high-throughput screening of potential cancer drugs and drug combinations on patient biopsy samples cultured ex vivo. These samples are grown as organoids in a 3D matrix that mimics the natural tumor environment.

Organoids were successfully generated from core needle biopsies of untreated breast tumors. These organoids were treated with the patient's prescribed neoadjuvant therapy, and early metabolic changes were quantified using OMI. Organoids grew from a variety of untreated breast tumor subtypes, and early metabolic changes could be resolved at the single-cell level after only 24 hours of treatment in vitro. In parallel, each patient's Residual Cancer Burden (RCB) score was quantified by a surgical pathologist after neoadjuvant treatment and served as gold standard validation of tumor drug response. Results from an early cohort of patients suggest that OMI could be used to predict patient clinical response to therapy. A linear combination of OMI variables measured in vitro in only 48 hours correlated strongly with patient RCB score (Pearson correlation coefficient=0.97, n=5). This methodology could allow oncologists to determine the ideal treatment regimen for their patients at the time of diagnosis.
Title: Quantitative assessment of tumor response to neoadjuvant chemotherapy in women with locoregional invasive breast cancer using Tc99m sestamibi molecular breast imaging - preliminary results

Gaiane M Rauch¹, Beatriz E Adrada¹, Cheenu Kappadath¹, Rosalind P Candelaria¹, Monica L Huang¹, Lumarie Santiago¹, Tanya Moseley¹, Marion E Scoggins¹, John D Knudtson¹, Benjamin P Lopez¹, Kenneth R Hess¹, Savitri Krishnamurthy¹, Stacy Moulder¹, Vicente Valero¹ and Wei Yang¹. ¹The University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Purpose: To report preliminary data in a pilot study evaluating the ability of Tc99m sestamibi Molecular Breast Imaging (MBI) to predict response and assess residual disease at the completion of neoadjuvant chemotherapy (NAC) in breast cancer patients.

Materials and Methods: Patients with localized, invasive breast cancer (T1-T4, N0-N3, M0) planned for NAC were enrolled in this prospective IRB approved clinical trial. All patients had digital mammography (DM), ultrasound (US), and MBI at baseline (T0), after 2 NAC cycles (T1), and at after NAC completion (T2). Tumor size and volume changes were compared with residual disease at surgery. MBI images were corrected for scatter and attenuation using a novel approach and regions of interest (ROI) were drawn over tumors to compute three quantitative MBI uptake metrics for correlation with pathologic response: tumor to background ratio (TBR), fractional activity uptake (FAU), and MBI-specific standardized uptake value (SUV). ROC analysis was performed.

Results: Patients (n=25) who completed NAC, had 75 imaging time points and had surgery, were included in this analysis. Median age was 49 years (range 31 -77). Eleven patients (11/25, 44%) had complete pathologic response (pCR). Absolute TBR values after 2 cycles (T1) and before surgery (T2) had highest correlation with pCR (AUC 0.81; 95% CI 0.63 to 0.99, p=0.01, and AUC 0.78; 95% CI 0.59 to 0.97, p=0.015, respectively). Change in SUV after 2 cycles, ∆SUV1 (T1-T0), (AUC 0.84; 95% CI 0.66 to 1.00, p=0.01) and change in SUV prior to surgery, ∆SUV2 (T2-T0) (AUC 0.80; 95% CI 0.60 to 1.00, p=0.02), were most predictive of pCR. Tumor size and volume showed modest specificity for detecting residual disease, and was highest for MBI (79%), followed by MMG (64%), and lowest for US (55%).

Conclusion: Quantitative MBI metrics show promise for the prediction of pCR in breast cancer patients undergoing NAC. Establishment of quantitative metrics for the early prediction of tumor response during NAC of breast cancer patients may provide an alternate to influencing NAC choice early in the management algorithm. Further investigation with a larger sample size is warranted.
**Title:** HER2 imaging by SPECT-CT using $^{111}$In radiolabeled pertuzumab-fab DOTAGA-conjugate: A proof of concept study in a preclinical model of breast cancer

Alexandra Oudot¹, Pierre-Simon Bellaye¹, Jean-Marc Vrigneaud¹, Olivier Raguin², Claire Bernhard³, Laure Dumont³, François Brunotte¹,²,³,⁴, Ariel Savina⁶, Fanny Bouquet⁶, Pierre Fumoleau¹ and Bertrand Collin¹,²,³, ¹Centre Georges-François Leclerc, Dijon, France; ²OncoDesign, Dijon, France; ³ICMUB UMR CNRS 6302, Dijon, France; ⁴NVH Medicinal, Dijon, France; ⁵Le2i UMR CNRS 6306, Dijon, France and ⁶Roche Institute, Boulogne-Billancourt, France.

**Body:**

*Introduction:* HER2 is positive in approximately 20-30% of all breast cancer and is associated with poor prognosis, higher mortality and higher metastatic incidence. Current diagnosis of HER2 expression relies on invasive methods requiring tissue biopsy which can lead to variable results due to inter/intra-metastatic and intratumoral heterogeneity in breast cancers. It has been recently demonstrated that HER2 molecular imaging based on pertuzumab, an antibody targeting HER2, could represent a more accurate non-invasive method to assess HER2 expression and evaluate its spatial and temporal heterogeneity.

**Aim:** In the present study, we aimed at developing radiolabeled pertuzumab Fab fragments for HER2 imaging. Tumor uptake of radiolabeled Fab was evaluated in an animal model of HER2 breast cancer by SPECT-CT, and the impact of trastuzumab on HER2 imaging was assessed. The objective of this study was to validate the feasibility of HER2 imaging with a pertuzumab-derived probe and to evaluate the possible translation of such a probe for clinical use in patients treated or not with anti-HER2 therapy.

**Methods:** Fab fragments of pertuzumab have been generated by papain digestion and bioconjugated with the bifunctional chelating agent DOTAGA for incorporation of Indium 111 to generate an HER2-specific probe for SPECT ($^{111}$In-DOTAGA-pertuzumab-Fab). The functionality of both radiolabeled Fab and whole pertuzumab was evaluated by *in vitro* assays using HER2-overexpressing human breast cancer cell line (HCC1954). Tumor uptake of pertuzumab-Fab and whole pertuzumab ($^{111}$In-DOTAGA-Pertuzumab) has been evaluated in Balb/c nude mice bearing BT-474 tumors with or without pre-treatment with trastuzumab for 24h.

**Results:** In the current study we demonstrate that Fab fragments of pertuzumab keep similar HER2 binding properties than whole pertuzumab and can therefore be a suitable HER2-targeted probe. *In vivo*, $^{111}$In-DOTAGA-pertuzumab-Fab showed better pharmacokinetics than whole pertuzumab with faster tumor uptake and blood clearance allowing faster imaging with better tumor/blood ratio. In addition, tumor uptake of $^{111}$In-DOTAGA-pertuzumab-Fab is not modified by pre-treatment with trastuzumab.

**Conclusion:** We assume that radiolabeled pertuzumab-Fab should be of great interest if the intention to treat is based on anti-HER2 monoclonal antibodies-based therapies rather than small molecules (*e.g.* lapatinib). Interestingly, pertuzumab has been shown to be also beneficial in combination with trastuzumab and chemotherapy in non-metastatic patients through its approval for the neoadjuvant treatment and very recently in the APHINITY clinical trial aiming for an approval in the adjuvant treatment of early breast cancer. These data suggest that pertuzumab will be extensively used in various HER-2 positive breast cancers underlining the interest to perform molecular imaging of HER2 expression as a predictive biomarker of efficacy.
**Title:** Predictive value of TILs in evaluation of 18F-FDG-PET in breast cancer

Tomoko Hirakata¹, Fujii Takaaki¹, Sasagu Kurozumi², Chika Katayama², Reina Oyayashi¹, Yuko Nakazawa¹, Naoko Tokuda¹, Keiko Yanai¹, Chikako Honda¹ and Hiroyuki Kuwano¹. ¹Gunma University, 3-39-22 Showa-machi, Maebashii, Gunma, Japan and ²Gunma University, 3-39-22 Showa-machi, Maebashii, Gunma, Japan.

**Body:** Background:
Several recent clinical studies have evaluated the prognostic and predictive importance of tumor-infiltrating lymphocytes (TILs) in breast cancer (BC). 18F-Fluorodeoxyglucose positron-emission (FDG-PET) CT was found to be effective for monitoring cancer cell viability of tissues and tumors, and it is used to evaluate the glucose metabolic rates of such tissues because most neoplasms have high glycolytic rates. FDG-uptake is also influenced by many factors, including inflammatory cells. In the present study, we investigated the relationship in evaluation of FDG-PET and degrees of TILs.

**Patients and Methods:**
Invasive carcinoma tissues of 100 breast cancer patients were examined. All patients underwent surgery. None of the patients had received preoperative chemotherapy. The proportional grades of stromal (Str)-TILs in surgical specimens were determined as follows: low (0-10%), intermediate (20-40%) and high (50-90%) grade, using the criteria of the International Working Group for TILs 2014 in breast cancer, using an immunohistochemical (IHC) method. The evaluation of PET was determined as follows, using standardized uptake value max (SUV max): low (<5) and high (>5). Estrogen receptor (ER), progesterone receptor (PgR), HER2, Ki67, and nuclear glade were also measured by IHC.

**Results:**
Among the 100 patients, range of age was 36 to 86 (mean 58) years. Twenty-seven patients were pre-menopausal, seventy-one were postmenopausal, and two were unknown. Invasive ductal carcinoma was diagnosed in 97 (97%) of the patients, invasive lobular carcinoma was diagnosed in 2 (2%), and others was 1 (1%). We divided the cases into three groups based on the degrees of TILs, low (57%), Intermediate (33%) and high (10%). We divided the cases into three based on the degrees SUV max, low (78%) and high (22%). High TILs group significantly showed higher SUV max than that of lower SUV max group (P=0.009). High TILs group had statistically higher rates of ER negative (P=0.001), PgR negative (P=0.002), HER2 positive (P=0.007), and nuclear grade 3 (P=0.013) than those of low TILs group.

**Conclusion:**
The proportional grades of stromal (Str)-TILs were associated with the degrees of SUV max in primary breast tumor in FDG-PET. High FDG uptake is predictive of poor prognosis and aggressive features in patients with breast cancer. From our findings, the evaluation of TILs is to be useful for predicting a better prognosis or therapeutic effect in cases with high FDG-uptake which indicate higher risk of recurrent disease.
Title: Second harmonic generation in combination with nuclear morphometry in the evaluation of DCIS

Collagen is a major extracellular matrix (ECM) constituent in normal breast and is extensively remodeled in breast carcinoma. Therefore, features of remodeled collagen in the stroma adjacent to ductal carcinoma in situ (DCIS) could indicate cancer progression. The major objective of this study is to identify potential tumor-associated collagen signatures unique to DCIS that will allow us to predict progression based on the collagen texture and nuclear morphology. In this present study, we develop two image analysis pipelines (SHG Texture Extraction and H&E Nuclear Morphology Extractor) to quantify 1) stromal changes, 2) collagen signatures and 3) nuclear morphology from normal breast to DCIS in order to predict local breast cancer recurrence.

Method: We used second harmonic generation (SHG) images and H&E to analyze collagen features and to study nuclear morphology using a data set of 336 patients (from which 310 normal and 327 DCIS regions were imaged). The 336 patients were a subset of patients with pure DCIS taken from a case-control study. Clinical-pathologic factors were associated with risk of subsequent ipsilateral cancer (DCIS or invasive). The SHG framework consisted of collagen segmentation using 1) adaptive thresholding and 2) morphological operations. The H&E framework consisted of nuclear segmentation using adaptive thresholding and a maker-controlled watershed algorithm; and nuclear feature extractions including intensity, texture and morphology. Overall, the SHG framework segments collagen regions and computes textural features specifically at collagen regions. Furthermore, the H&E framework segments nuclei and computes nuclei morphology and textural features. These features were used in L1-regularized logistic regression to construct classification models to discriminate normal vs DCIS regions; and to distinguish regions from DCIS patients with vs. without local recurrences.

Results: In first experiment, we performed L1-regularized logistic regression to construct a classification model to discriminate normal vs DCIS regions. Our results suggest that using only SHG collagen features, this logistic model selected 19 significant features to build a classification model that achieved area under curve (AUC) 90% and accuracy 83% using 5-Fold cross validation. When H&E nuclei features are used, the logistic model selected 88 significant features and achieved AUC 91% and accuracy 86%. By combined both SHG and H&E features, the model achieved classification AUC 93% and accuracy 88%. By using L1-regularized logistic model with combined significant SHG and H&E features, we achieved AUC 59% with an accuracy of 61% for DCIS and recurrent DCIS regions.

Conclusions: Our study suggests that SHG and nuclear morphology features extracted from H&E can improve the classification of normal and DCIS regions. Overall, these results suggest that second harmonic generation and H&E nuclear morphology analysis could aid in the assessment of prognosis and risk of progression to invasive breast cancer.
Title: Evaluation of pathological malignancy grade and neoplastic progress of breast cancer using dedicated breast positron emission tomography

Norio Masumoto¹, Takayuki Kadoya¹, Mai Nishina¹, Yuri Kimura¹, Eri Suzuki¹, Satoshi Sueoka¹, Noriko Goda¹, Shinsuke Sasada¹, Keiko Kajitani¹, Akiko Emi¹, Rumi Haruta², Tsuyoshi Kataoka² and Morihito Okada¹. ¹Hiroshima University, Hiroshima and ²Hiroshima University Hospital, Hiroshima.

Body: Background: Dedicated breast positron emission tomography (DbPET) provides detailed high resolution images of the breast and enables quantitative assessment using standard uptake values (SUVs). We aimed to determine whether DbPET can predict the pathological malignancy grade and neoplastic progress of breast cancer compared with whole body (WB) PET.

Methods: We investigated 196 consecutive patients with invasive breast cancer who underwent concurrent Db- and WB-PET from January 2016 to March 2017. All Db- and WB-PET were quantified based on SUVs. We also investigated pathological features of breast cancer who had a ring-like uptake (RU) without central FDG accumulation on DbPET.

Results:

The associations between the SUVs for DB- and WB-PET and the pathological factors in breast cancer

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>WB PET</th>
<th>p</th>
<th>DbPET</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>all</td>
<td>3.6 ± 3.4</td>
<td></td>
<td>9.4±7.9</td>
<td></td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤2.0 cm</td>
<td>2.2±1.6</td>
<td>&lt;0.001</td>
<td>6.5±5.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&gt;2.0 cm</td>
<td>5.5±4.1</td>
<td></td>
<td>13.3±9.2</td>
<td></td>
</tr>
<tr>
<td>LN</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>3.1±3.3</td>
<td>&lt;0.001</td>
<td>8.4±7.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Positive</td>
<td>4.9±3.2</td>
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<td>11.8±7.7</td>
<td></td>
</tr>
<tr>
<td>NG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 or 2</td>
<td>2.4±2.1</td>
<td>&lt;0.001</td>
<td>6.6±5.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3</td>
<td>5.1±4.0</td>
<td></td>
<td>12.7±8.7</td>
<td></td>
</tr>
<tr>
<td>Ki67</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 20</td>
<td>1.8±1.1</td>
<td>&lt;0.001</td>
<td>5.2±3.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>≥ 20</td>
<td>4.4±3.7</td>
<td></td>
<td>11.4±8.6</td>
<td></td>
</tr>
<tr>
<td>ER</td>
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</tr>
<tr>
<td>positive</td>
<td>3.4±3.3</td>
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<td>8.8±7.6</td>
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</tr>
<tr>
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<td>5.2±3.6</td>
<td></td>
<td>13.5±8.6</td>
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</tr>
<tr>
<td>HER-2</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>positive</td>
<td>4.6±3.1</td>
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</tr>
<tr>
<td>Sub type</td>
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<td>vs Luminal A</td>
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<td></td>
</tr>
<tr>
<td>Luminal A</td>
<td>1.8±1.1</td>
<td>5.2±3.3</td>
<td></td>
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</tr>
<tr>
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<td>10.1±8.5</td>
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<tr>
<td>HER2</td>
<td>4.6±3.1</td>
<td>&lt;0.001</td>
<td>11.8±7.6</td>
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</tbody>
</table>
summarizes the association between SUVs for Db- and WB-PET and pathological factors in breast cancer. SUVs on PET were significantly higher for the tumor size of >2.0 cm than for tumor size ≤2.0 cm (p<0.001), for LN-positive than for LN-negative (p<0.001), for NG3 than for NG1-NG2 (p<0.001), for higher Ki67 expression than for lower Ki67 expression (p<0.001), and for ER-negative than for ER-positive (WB-PET, p=0.02; DbPET, p=0.006). SUVs were significantly lower for Luminal A than for Luminal B, HER2, and triple-negative cancer (p<0.001 for all three). SUVs for DbPET was significantly higher for HER2-positive than for HER2-negative (p=0.02).

The association between SUVs for breast cancer with and without RU on DbPET

<table>
<thead>
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<th>Characteristic</th>
<th>RU(-), n</th>
<th>RU(+), n</th>
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</thead>
<tbody>
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<td>all</td>
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<tr>
<td>Tumor size</td>
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<td></td>
</tr>
<tr>
<td>≤2.0 cm</td>
<td>109</td>
<td>5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&gt;2.0 cm</td>
<td>64</td>
<td>18</td>
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</tr>
<tr>
<td>LN</td>
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<td></td>
</tr>
<tr>
<td>Negative</td>
<td>129</td>
<td>9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Positive</td>
<td>44</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>NG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 or 2</td>
<td>100</td>
<td>7</td>
<td>0.02</td>
</tr>
<tr>
<td>3</td>
<td>73</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Ki67</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 20</td>
<td>61</td>
<td>3</td>
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</tr>
<tr>
<td>≥ 20</td>
<td>112</td>
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<td></td>
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<tr>
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<tr>
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<td>152</td>
<td>19</td>
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</tr>
<tr>
<td>negative</td>
<td>21</td>
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<td></td>
</tr>
<tr>
<td>HER-2</td>
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</tr>
<tr>
<td>positive</td>
<td>26</td>
<td>2</td>
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<td>147</td>
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<td>vs Luminal A</td>
</tr>
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<td>3</td>
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<td>Luminal B</td>
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<td>15</td>
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<td>HER2</td>
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<td>0.81</td>
</tr>
<tr>
<td>Triple negative</td>
<td>14</td>
<td>3</td>
<td>0.04</td>
</tr>
</tbody>
</table>

summarizes the association between SUVs for breast cancer with and without RU on DbPET. SUVs for breast cancer with RU on DbPET were significantly higher for the tumor size of >2.0 cm than for tumor size ≤2.0 cm, for LN-positive than for LN-negative (p<0.001), for NG3 than for NG1-2 (p=0.02), and for higher Ki67 expression than for lower Ki67 expression (p=0.03). SUVs were significantly lower for Luminal A than for Luminal B (p=0.02) and triple-negative cancer (p=0.04).

Conclusions: SUVs for DbPET were equal or superior to WB-PET in predicting the pathological malignancy grade and neoplastic progress in tumors. Furthermore, the presence of RU on DbPET can provide excellent predictive value for high-grade malignancy and might help to determine appropriate therapeutic strategies.
**2017 San Antonio Breast Cancer Symposium**

**Publication Number:** P5-02-04

**Title:** Opto-acoustic imaging of breast masses: Correlation with breast biopsy prognostic indicators

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**Body:** Purpose: The Imagio™ OA/US breast imaging system, a diagnostic opto-acoustic (OA) imaging device bearing the CE Mark, is in the U.S. FDA Premarket Approval process. OA/US provides both functional (relative oxygenation/de-oxygenation) and anatomic (angiogenesis) information that is co-registered and temporally interleaved in real time with gray-scale ultrasound that may improve discrimination between benign and malignant masses. We recently reported correlation studies demonstrating tumor-zone specific OA attributes in histopathologic grade I versus grade III malignancies. The relationship between OA attributes (individual feature scores or summed feature results) and pathologically-determined prognostic markers (PDPM) in malignant lesions is the subject of this report.

**Materials and Methods:** In this HIPAA-compliant, IRB-approved prospective multi-center trial across 16 U.S clinical sites: 1,808 masses in 1,739 subjects assessed as BI-RADS 3, 4 or 5 were imaged with OA/US. Of these, 655 were invasive malignancies and the subject of this analysis. Each mass was scored by 8 blinded readers on 3 internal zone features of the tumor nidus and 2 external features (0-5, 6) of the tumor boundary and peripheral zones (OA attributes). Pathologic diagnoses were confirmed by an experienced central breast pathologist blinded to the OA assessment. Tumor histologic classification and grading was performed in all subjects. Evaluation of tumor estrogen receptor (ER) and progesterone receptor (PR) were performed at each site by immunohistochemistry (IHC) and was reported as percent of tumor cells expressing the receptor or, as positive if greater than 1%. Tumor HER2-neu expression was reported by IHC as 0, 1+ (negative, not over-expressed), 2+ (indeterminate) and 3+ (over-expressed). All 2+ results reflexed to fluorescence in-situ hybridization (FISH) and reported as over-expressed or not over-expressed. Tumor Ki-67 expression was evaluated with IHC and reported as percent of tumor cells positive for the antigen. Statistical analysis of categorical measures of PDPM is in process and will be performed using a two-way Analysis of Variance (ANOVA) and Tukey HSD (honest significant difference) test for pairwise comparisons. This ANOVA will be repeated for each PDPM to test which specific PDPM sub-categories are related to OA attributes. Correlation coefficients will be generated for PDPM that are continuous, not categorical. All statistical testing will be done at a 5% significance level.

**Results:** A total of 655 invasive and 22 DCIS were scored for internal (nidus) and external (boundary and periphery) OA attributes and compared with PSBC as defined by ER, PR, Her2 and Ki-67 expression. Of these, 108 were Luminal-A (LA), 153 Luminal-B (LB), 80 Triple-negative (TN), 23 Her2-enriched (HER2) and 314 unclassified (including 22 DCIS). OA attributes differentiated LA (99% CI 2.8,3.1) from TN (99% CI 3.1,3.4), p=0.027 and HER2 (99% CI 3.1,3.6), p=0.036. OA features strongly suggested LA vs. LB (99% CI 3.1,3.3) subtype, p=0.060. LB vs.TN(p=0.59) and HER2(p=0.41) were non-significant. TNBC vs. HER2 was p=0.62.
Title: A genomic ruler to assess oncogenic transition between breast tumor and cancer-free stroma

Robert J Schneider¹, Shubhada Dhage¹, Amanda Ernlund¹, Kelly Ruggles¹, Deborah Axelrod¹, Russell Berman¹ and Daniel Roses¹. ¹New York University School of Medicine, New York, NY.

Body: Abnormal gene expression changes are promoted by tumors in the surrounding “normal” stromal tissue, which remains poorly characterized due to the use of opportunistic biopsies at random distances from the tumor. We therefore sought to connect the 3-dimensional spatial relationship of the breast tumor, the stromal landscape and changes in cancer promoting and homeostatic tissue-normalizing stromal gene expression. We investigated the extent to which breast tumor gene expression alters breast stromal gene expression in 33 women undergoing mastectomy for primary invasive ductal carcinoma. Gene expression changes in histo-pathologically normal breast tissue were obtained at serial distances from the tumor in 3-dimensions in the breast and subjected to genome-wide gene expression analysis in multiple dimensions using novel bioinformatic approaches based in part on 3-dimensional facial recognition algorithms.

Methods: All patients underwent a mastectomy with lymph node sampling. None of the patients had multi-centric disease or underwent neo-adjuvant therapy. 57% of patients were stage II, 43% were stage III, and 50% of all tumors were poorly differentiated. Bulk tissue was isolated from mastectomy specimens immediately after surgery, initiating at the gross tumor edge, then every 5 mm up to 20 mm in the longest 3-dimensional axis. All stromal specimens were pathologist verified as devoid of cancer cells. RNA was purified, subjected to genome-wide transcriptional analysis using limma and Non-Negative Matrix Factorization methods, to reduce the dimensionality of biological data and identify molecular signatures of different tissue compartments composed of various cell types in multi-dimensional space.

Results: Surprisingly, a discreet tumor-like signature was identified in the stroma at all distances and in all dimensions from the tumor at every point sampled in the breast. Stroma within 5 mm of the tumor-free margin displayed the most highly tumor-like signature, but at any distance stromal gene expression was enriched in cancer-promoting pathways involved in disruption of basement membrane, cell migration, PI3K-AKT signaling, immune evasion and angiogenesis. With distance from tumor, gene expression transitioned to include a countering, homeostatic normalizing signature, enriched only at distances far from tumor. In all dimensions, even in other quadrants of the breast, “normal” stroma continued to express cancer-supporting pathways unrelated to desmoplastic responses or immune infiltration, but when far from the tumor border, also expressed normalizing homeostasis and tumor suppression genes.

Conclusions: Our results indicate that the dynamics of gene expression flux in the stroma likely co-determine the fate of treatment and recurrence beyond that of the elimination of cancer cells and cancer stem cells, additionally determining reversion to normality or transition to neoplasia in morphologically normal stromal breast tissue. Our identified tumor-like stromal gene expression signature may lead to improved treatment, new treatment markers and improved risk assessment, possibly more accurately and individually describing risk and treatment outcome.
Title: Three-dimensional (3D) imaging of biomarkers in human core needle biopsies of normal and cancerous breast tissue

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Body: Background: The tumor microenvironment is spatially and compositionally very heterogenous, which introduces great challenges to characterize the underlying factors using standard 2D diagnostic methodologies. Capturing high resolution 3D quantitative biomarker data, while simultaneously preserving morphology of the tumor microenvironment, could lead to a better understanding of key spatial relationships and may lead to better prognostic and predictive clinical outcomes. In this study, we utilized a novel technique, CLARITY, to transform core needle biopsies from patients with breast cancer, into optically transparent tissues, followed by multiplex immunostaining and 3D imaging of molecular markers. This data was compared to the conventional methods of immunohistochemistry and immunofluorescence staining on FFPE thin sections.

Methods: Formalin-fixed (less than 24 hours) human breast cancer core needle biopsy tissue pairs (tumor and adjacent normal) were obtained from patients undergoing excisional surgery. Tissues were subsequently embedded in 4% paraformaldehyde containing a 4%/0.05% ratio of acrylamide/BIS for 48 hours, and polymerized to form an intact hydrogel/tissue matrix. The samples were sectioned to a thickness of 200µm, 400 µm, and 1000µm and lipid-cleared in a solution of 0.2M borate buffer containing 8% SDS, pH 8.5 at 45°C. The tissues were then immunostained for various cellular markers (Pan-cytokeratin, Her2, CD3, CD31) and a nuclear marker (Ki67) and counterstained with DAPI. Samples were refractive index matched prior to 3D imaging on a Leica SP8 laser scanning confocal microscope or a LaVision BioTec Ultramicroscope II, light sheet microscope.

Results: During the process, the samples remained intact and the cellular morphology was well preserved suggesting that a pre-fixation step following by hydrogel embedding/Immunostaining was feasible. The average passive lipid-clearing time for breast cancer core needle biopsy tissue was 5-20 days depending on the size of the tumor. The majority of the samples reached visual optical transparency, with the exception of some regions that contained heavy fibrotic tissue. Preliminary results demonstrated that specific staining of various cellular and nuclear markers was successful as evidenced by 3D imaging depth up to 1000 µm. As compared to the images obtained from 2D thin sections, the CLARITY procedure followed by 3D imaging yielded significant imaging depth, with the potential to greatly enhance the understanding of the heterogeneity of the tumor microenvironment.

Conclusion: This is the first study demonstrating that other than fresh or frozen tissues, pre-fixed clinical tissue from patients with breast cancer, can be successfully processed by the CLARITY methods and 3D imaged, indicating the potential power of the technique for core needle biopsy tissue processing and in the identification of biomarkers based on tumor cell heterogeneity.
Title: Cytotoxic t-lymphocyte density increased within the tumor immune microenvironment of patients with breast cancer following treatment with Akt inhibitor MK-2206

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Body: Background: The PAM (PI3K/Akt/mTOR) signaling pathway has been implicated in the oncogenesis of multiple solid malignancies, including breast cancer (BC). Tumor infiltrating lymphocytes (TILs) found within the tumor immune microenvironment (TME) are both prognostic of overall survival as well as predictive of response to neoadjuvant chemotherapy in BC. Our aim is to characterize the TME in a series of patients with operable stage I-III BC treated with MK-2206, an allosteric Akt inhibitor as part of a presurgical, window of opportunity, trial. In our presurgical trial (clinicaltrials.gov #: NCT01319539), patients received 2 doses of MK-2206 with first dose at day -9 and second at day -2 from surgery.

Methods: Quantitative multiplex immunofluorescence (qmIF) was performed using immune biomarkers (DAPI, CD3, CD8, CD4, FOXP3, CD68, Pancytokeratin) on full section (4uM) tissue slides from core biopsy specimens and postsurgical specimens of 10 patients - 5 patient treated with MK-2206, and 5 prospectively collected untreated controls. Images were taken using Vectra, a novel pathology workstation and analyzed using inForm software to perform cell classification and phenotyping. Student T- test was used to compare biomarker changes before and after MK-2206.

Results: Preliminary analysis demonstrates that patients treated with MK-2206 exhibited a significantly increased median cytotoxic T-cells (CD3CD8+) density, as compared to the matched control cohort (87% vs.0.2%, p < 0.05). We did not identify a change in macrophage (CD68) or T helper/T reg (CD4/CD4FOXP3+) density following MK-2206 treatment in this small cohort. Proximity comparison, using nearest neighbor analysis was used to assess for potential impact of therapy on interactions between CD3CD8+ cells and BC cells. The median distance from CD3+CD8+ cells to nearest neighboring tumor cell was determined in both the pre and post tissues specimens for both groups. In patients treated with MK-2206, a 12.5% reduction in median distance was observed between CD3+CD8+ cells and tumor cells following treatment, suggestive that the increased effector T cells are not relegated to the periphery. This observation was not seen in the pre and post samples of the control cohort.

Conclusions: In our study, we found that presurgical Akt inhibition lead to a significant increase in the cytotoxic T-cell population which was in similar proximity, if not closer, to tumor cells as compared to matched controls. Limitations of this exploratory study include a small patient cohort size and use of a single pathology evaluation technique. We are currently expanding our characterization of the TME with a more comprehensive myeloid panel as well as performing additional tissue analysis to validate our findings. At present, there are currently FDA approved therapies, as well as agents in clinical development that exert antineoplastic activity through the PAM pathway. Investigations that endeavor to understand the impact of these therapies on the TME may lead to both an increased understanding of the bioactivity of these agents and potentially identify aspects of the immune response which can be exploited by future immunotherapy therapeutics.
Title: Fibroblast-kinome silencing identifies key microenvironment mediators implicated in TNBC invasion

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Body: Background: Deregulation of kinases lead to tumor growth and malignant progression by driving many of the hallmarks of cancer. As a result, pharmaceutical intervention targeting aberrant kinase signaling represents the main therapeutic approach in cancer management. However, innate or acquired resistance develops emphasizing the need for the identification of new druggable targets.

Despite mounting evidence for the role of the tumor microenvironment (TME) in metastatic progression, little is known about how stromal cells influence the behavior of cancer cells and how they affect their response to target therapy. Fibroblasts are prominent cells in the TME and induce beneficial and adverse effects in pre-metastatic progression. Therefore, shedding light on the signaling pathways involved in fibroblast-tumor cells cross-talk could lead to the identification of new strategies for the prevention and treatment of triple negative breast cancer (TNBC), which is associated with a worse prognosis amongst the breast cancer subtypes and still lacks effective therapy.

Aims: Considering the above and the fact that research/clinical trials to date have mainly focused on targeting cancer cells, the aims of this study are to: i) identify fibroblast-expressed kinases that modulate TNBC cells' invasion and ii) elucidate the mechanism of action via which these kinases can promote/reduce TNBC invasion.

Methods/Results: Co-culturing of MDA-MB-231 with Human-Mammary-Fibroblasts (HMF) and normal Lung Fibroblasts (MRC-5) revealed changes in gene/protein expression levels in cancer cells, supporting the hypothesis of a cross-talk between different cell types.

To identify the role of kinases involved in tumor-stroma cross-talk, fibroblasts (HMF/MRC-5) were transfected with a pool of 3 siRNAs/gene targeting each of the 710 kinases. Following, fibroblasts were co-cultured with MDA-MB-231 in 3D-spheroids and the invasion potential was assessed by confocal microscopy. Spheroids pictures taken at different time-points were analyzed and results were expressed as changes in spheroid diameter ($\Delta$ of the non-targeting/scrambled siRNA).

The $\Delta$-value of each silenced kinase ($\Delta_K$) was compared with the $\Delta$-value of the control ($\Delta_CT$), at different time-points, to obtain a $\Delta\text{Ratio} = \frac{\Delta_CT}{\Delta_K}$; kinases with $\Delta\text{Ratio}$ between 0.7-2 were considered uninfluential on the invasiveness of MDA-MB-231, while kinases with $\Delta\text{Ratio} < 0.7$ were classified as "invasion-promoting kinases", and kinases with $\Delta\text{Ratio} > 2$ were classified as 'invasion-inhibiting kinases'. We identified 8 kinases (in HMF and MRC-5), whose silencing decreased the TNBC invasion rate, suggesting a pro-invasive role of these proteins.

Further analyses (RNA-sequencing/SILAC) demonstrated distinct differences in TNBC cells' genomic/proteomic signatures when co-cultured with knocked-out fibroblasts for these "invasion-promoting kinases". In vitro/in vivo data on the cross-talk pathways mediated after inhibition of these kinases, between fibroblasts and TNBC cells, will be presented.

Conclusion: Our experimental approach/results provide new information in understating the cross-talk between tumor and stroma and identified key fibroblasts-expressed kinases implicated in TNBC invasion, which could provide the basis for new therapeutic strategies.
Title: Sphingosine-1-phosphate produced by sphingosine kinase 1 and exported via ABCC1 shortens survival of mice and humans with breast cancer

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Body: Background: Sphingosine-1-phosphate (S1P), a bioactive sphingolipid mediator that is generated by sphingosine kinase 1 (SphK1) when it is phosphorylated (pSphK1) inside cells, has been implicated in regulation of many process important for breast cancer progression. Previously we have shown that S1P is exported out of human breast cancer cells by ATP-binding cassette (ABC) transporter ABCC1, but not by ABCB1, both known multidrug resistance proteins that efflux chemotherapeutic agents. However, the pathological consequences of these events to breast cancer progression and metastasis have not been elucidated. Here, we report that high expression of ABCC1, but not ABCB1, is associated with poor prognosis in breast cancer patients via exporting S1P.

Materials and methods: Microarray based gene expression data of 2509 patients associated with their survival were obtained from METABRIC database. Single gene survival analysis based on expressin of SphK1, and dual ABCC1 or ABCB1 and SphK1 survival analyses were performed. For protein analyses, tissues were obtained from 275 patients with stage 1-3 breast cancers treated in Yokohama City University Medical Center in Japan between 2006 and 2008. The expression of pSphK1 was analyzed by immunohistochemistry and investigate the relationship with clinicopathological findings. For in vitro and in vivo experiments, breast cancer cell lines were transfected by ABCB1, ABCC1 or vector transiently or stably. BALB/c nu/nu mice and BALB/c mice were used for in vivo experiments. S1P was measured by LC-ESI-MS/MS.

Results: SphK1 expression significantly associate with worse overall survival (median survival of 124 months with high SphK1 expression compared to 163 months for patients with low SphK1 expression, p=0.0014). Although patients with high ABC1 expression had only a slightly worse overall survival of 150 months, those with high levels of both SphK1 and ABCC1 had much worse prognosis with median overall survival of 114 months (p < 0.0068). Such association was not observed with ABCB1 expression. The frequency of strong pSphK1 protein expression was higher in HER2 enriched or TNBC than in Luminal. pSphK1 was more prevalent and increased in a larger tumors and in tumors from patients with lymph node metastases. Patients with breast cancers that express both pSphK1 and ABCC1 proteins have significantly shorter disease free survival. Overexpression of ABCC1, but not ABCB1, in human MCF7 and murine 4T1 cells enhanced S1P secretion, proliferation and migration of breast cancer cells. Implantation of breast cancer cells overexpressing ABCC1, but not ABCB1, into the mammary pad markedly enhanced tumor growth, angiogenesis and lymphangiogenesis with concomitant increases in lymph node and lung metastases as well as shorter survival of mice. Interestingly, S1P exported via ABCC1 from breast cancer cells upregulated transcription of SphK1 and its own formation.

Conclusions: Our findings suggest that production and export of S1P via ABCC1, but not ABCB1, is associated with worse overall and disease free survival of breast cancer patients and that S1P axis play a role in aggressive biology of breast cancer progression and metastasis.
Title: Novel targets of breast cancer associated with inflammatory tumor microenvironment


Body: Breast cancer affects one in eight women in the USA. Considerable progress in the identification of genetic lesions and their modulation has resulted in newer therapies making breast cancer a manageable disease. However, triple negative breast cancer is still difficult to treat and warrants a search for newer targets. To this end, we focused our attention towards the modulation of the breast cancer epithelium by other cell types such as the endothelial cells and the macrophages. The migratory macrophages and the estrogen sensitive migratory endothelial progenitor cells (EPCs) constitute the cellular milieu within the tumor microenvironment which continuously modulates breast cancer epithelium. We analyzed the interactions of the breast cancer cell lines (MCF-7 and MDA-MB-231) with the highly proliferative human umbilical cord derived CD133+/CD34+/VEGFR-2+ EPCs and M1 polarized macrophages (activated THP-1 cell line) in two separate in vitro studies. The readouts were cell proliferation, changes in epithelial to mesenchymal transition (EMT), and cellular differentiation. We observed morphological and cellular growth changes in the EPCs on treatment with conditioned medium (CM) generated from breast cancer cells, consistent with vasculogenesis and in vitro tubulogenesis. Both, MDA-MB-231 and MCF-7 CM, treatments resulted in enhanced EPCs proliferation and differentiation. However, the differentiation patterns were distinct, with MCF-7 CM increasing the number of cell clusters, whereas MDA-MB-231 CM increasing the number of adherent spindle shaped cells. The paracrine interaction was also assessed with M1 polarized macrophages. We observed decreased cell viability in MCF-7 and MDA-MB-231 cells following activated THP-1 CM and exosome treatments. Analysis of exosomes from activated THP-1 indicated an upregulation of 13 miRNAs compared to unactivated THP-1. The miRNA hsa-miR-146a-5p had the highest upregulation (44 fold increase). This specific miRNA has been observed in senescent cell and it inhibits cell proliferation, suggesting a possible mechanism for exosome-associated growth inhibition. The analysis of the paracrine interactive mediators between breast cancer cells, EPCs, and M1 polarized macrophages is likely to yield viable novel clinically translatable therapeutic targets.
Probing breast cancer cell activation in response to extracellular cues using well-defined synthetic microenvironments

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Interactions between breast cancer cells and their microenvironments are essential in tumor growth, metastasis, and recurrence. The tumor stroma undergoes constant structural changes, including degradation, redeposition, and crosslinking of collagens with gradients in matrix stiffness and composition that drive invasion and metastasis. At metastatic sites, similar remodeling events that occur with injury and aging are hypothesized to promote reactivation of dormant tumor cells in recurrence. Approaches are needed for testing hypotheses about pivotal cell-matrix interactions in the progression of breast cancer for identifying key regulators and improving treatment strategies.

In this work, we have designed well-defined materials to mimic key aspects of tumor microenvironments toward studying such complex phenomena in vitro. Specifically, we have created synthetic extracellular matrices with well-defined biophysical and biochemical properties that enable three-dimensional (3D) culture of breast cancer cells and niche cells over weeks. A bioinert polymer, multiarm poly(ethylene glycol) functionalized with thiols (Mₙ ~ 20 kDa), was reacted with integrin-binding and cell-degradable peptides decorated with one and two allyloxycarbonyl protecting groups, respectively, by rapid light-triggered thiol-ene polymerization for independent control of matrix density and composition. The elasticity, or 'stiffness', of these matrices has been tuned to mimic a variety of soft tissues (Young's modulus E~0.5-20 kPa), from healthy and cancerous mammary tissues to metastatic site bone marrow and lung tissues. Further, the biochemical content has been tuned with receptor-binding peptides derived from laminin (IKVAV, laminin receptor), collagen ((POG)₃POGFOGER(POG)₄, α₂β₁ and α₁β₁), and fibronectin/vitronectin (RGDS, αVβ₃ and α5β₁ amongst others) and a crosslinking peptide derived from collagen (GPQG↓IWGQ, degraded MMP-1, -2, and -9 amongst others).

We hypothesized that a microenvironment rich in collagen and fibronectin/vitronectin, mimicking aspects of remodeled tissues, would activate breast cancer cells relative to a laminin-rich epithelium-like microenvironment, building upon seminal studies in naturally-derived matrices and in vivo. To test this, we cultured breast cancer cells of different metastatic potential (estrogen receptor positive [ER+, T47Ds] and triple negative [ER-, MDA-MB-231s]) within different matrix densities and compositions. Both cell types exhibited high viability (> 90%), and cell activation in response to different matrix compositions was assayed by examining proliferation (metabolic activity, Ki-67, cell/cluster number and volume) and phenotype (morphology; E-cadherin, vimentin). Increased matrix density decreased elongation of ER- cells and proliferation of both cell types. Increased collagen content increased the proliferation of the ER+ cells and proliferation and elongation of ER- with mass and stellate morphologies, respectively, like observed in naturally-derived matrices. These studies demonstrate a new tool for controlled 3D culture of breast cancer cells relevant for both fundamental and applied research, with on-going investigations incorporating niche cells and triggered matrix changes.
Body: Introduction: Anthracycline-based chemotherapy regimens have been shown to increase risk of cardiac toxicity and other side effects especially in combination with HER2-targeting agents such as trastuzumab. Identification of biomarkers that can predict similar patient benefit in the context of targeted therapy between anthracycline and non-anthracycline-based regimens is attractive for personalized care. Histology-based assessment of tumor infiltrating lymphocytes (TILs) as a surrogate of the host immune response has been shown to be prognostic and potentially chemopredictive in triple-negative and HER2-positive breast cancers; however, the inter-play of TILs, tumor cells, other microenvironment mediators, their spatial relationships, quantity, and other image-based features have yet to be determined exhaustively and systemically. In anticipation of analyzing these aspects in the context of chemo and targeted therapy response in patient sample cohorts, we developed a digital pathology image analysis algorithm to identify tumor, stromal, and lymphocyte cells and acquire respective histology-based image features from hematoxylin and eosin (H&E) stained slides. Materials and Methods: An automated method involving cell detection, cell segmentation, feature extraction (capturing both local features and global context based features) and supervised machine learning (using a multi-class random forest based classifier, where a 3-class problem is represented using 3 1-vs-1 binary classifiers) were used to classify individual cells into the following 3 categories: tumor cells, stromal cells, and lymphocytes. Cell classification was compared against manually determined ground truth from three pathologists using simple confusion matrices. Results: From six H&E breast cancer cases, two pathologists manually and independently annotated the same tumor cells (6,458), lymphocytes (2,491), and stromal cells (744) in fourteen field-of-views (~ 0.3 mm² in size). Manual concordance of tumor cells (99.4%, 1434/1442), lymphocytes (80.0%, 680/849), and stromal cells (68.8%, 53/77) between two pathologists was moderate to high. Comparing only cells where two pathologists agreed (4,736) and an independent set of single cell annotations (547) from a third pathologist, image analysis classification showed high concordance for tumor cell (92.9%, 1107/1191), lymphocyte (90.4%, 572/636), and stromal cell (94.3%, 66/70) classification categories. Approximately 242 image features grouped into 22 unique data families were extracted from each cell analyzed. Conclusion: A H&E-derived TILs image analysis algorithm with associated feature extraction is feasible with preliminary findings of accurate cell classification. This tool will continue to be refined in anticipation of analysis in patient outcome cohorts.
Title: Breast cancer promotion by IL-6-mediated cross-talk between human preadipocyte and breast ductal carcinoma *in situ*

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Body: While the role of adipocyte, one of the major components of the breast microenvironment, has been well documented in breast cancer progression, the preadipocytes function to favor early-stage breast cancer progression at the molecular level is not fully elucidated. To delineate the role of human preadipocyte (hPreAd) on breast cancer progression, we investigated a cross-talk between human breast ductal carcinoma in situ (DCIS) and hPreAd in in vivo culture and in vivo tumor model. On Co-culture system with DCIS cell line MCF10DCIS.com and primary hPreAd, proliferation, migration and invasion of MCF10DCIS.com was promoted by hPreAd. Conditioned medium (CM) of hPreAd activated signaling molecules (AKT1, ERK1/2, mTOR, FAK and STAT3) in MCF10DCIS.com. hPreAd expressed fibroblast specific protein 1 (FSP1) and alpha-smooth muscle actin (α-SMC), and increased IL-6 secretion by co-culture or MCF10DCIS.com CM, whereas adipocyte differentiation of hPreAd was suppressed by co-culture. A neutralizing antibody (NAb) of IL-6 or IL-6 receptor suppressed the MCF10DCIS.com proliferation and migration enhanced by co-culture of hPreAd. In xenograft tumor model, tumor growth of MCF10DCIS.com was enhanced by co-injection with hPreAd. IL-6 NAb had minimal effect in xenografts with MCF10DCIS.com alone; however, in models co-administered with hPreAd, IL-6 NAb had more potent effects on tumor inhibition. Taken together, our study suggests that IL-6-mediated cross-talk between preadipocyte and breast DCIS can exert the breast cancer progression at early-stage. Deciphering the cellular and molecular mechanisms behind the preadipocyte-breast DCIS crosstalk might provide new targets for improving diagnosis/prognosis and for the design of innovative therapeutic strategies.
**Title:** IL-6 and CCL5 secretion by adipose-derived stem cells and the breast tumor microenvironment

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**Body:**

**Introduction:** Obesity is a key factor in promoting aggressive breast cancers in women. In previous studies, we found increased production of IL-6 and CCL5, common pro-inflammatory cytokines, in co-cultures of adipose stem cells and triple negative breast tumor cells. When we probed The Cancer Genome Atlas (TCGA) for triple negative breast cancer, we discovered that CCL5 overexpression was associated with improved survival. This finding contradicts the majority of *in vitro* studies regarding the role of CCL5 in the breast tumor microenvironment; the literature suggests that CCL5 promotes tumor metastatic ability. Furthermore, TCGA data did not indicate a significant correlation between IL-6 production and survival outcomes. It remains unclear whether CCL5 and IL-6 are produced by adipose stromal cells or cancer cells within the tumor microenvironment. We predict that the primary source of CCL5 and IL-6 is from adipose stromal cells. However, the production of these cytokines may be altered when exposed to tumor-secreted factors. **Methods:** Adipose-derived stem cells (ASC) and preadipocytes differentiated from ASCs (Pread(A)) were treated with the conditioned media of triple negative breast tumor cells (MDA-MB-231) and luminal A breast tumor cells (MCF-7). In addition, MDA-MB-231 and MCF-7 cells were treated with the conditioned media of each adipose stromal cell type. After 72 hours of treatment, the media harvested from each cell type was analyzed for secreted IL-6 and CCL5 proteins. **Results:** IL-6 and CCL5 levels in the conditioned media of ASCs treated with MDA-MB-231 or MCF-7 cells were significantly lower (p<0.05) when compared to the media of ASCs alone. The reverse occurred when tumor cells were provided conditioned media from adipose progenitor cells. When both breast tumor cell lines were exposed to conditioned media from ASCs and Pread(A), the secretion of IL-6 and CCL5 increased significantly (p<0.05). The conditioned media of Pread(A) cells treated with breast tumor cells were lower than untreated cells, however, this decrease in cytokine production was not significant. **Conclusions:** This study suggests that IL-6 and CCL5 secretion by adipocytes is modified by the presence of breast tumor cells. The significant decrease in IL-6 and CCL5 secretion from both adipose-derived stem cells and preadipocytes in the presence of tumor may suggest an attempt by the tumor to inhibit an inflammatory response by adipose stromal cells while increasing its own IL-6 and CCL5 production. Although the human genome data indicates that CCL5 and IL-6 provide a survival benefit *in vivo*, laboratory *in vitro* studies thus far have failed to mimic the observed clinical responses. Further studies will investigate the clinical relevance of CCL5 and IL-6 receptors in breast cancer. (Supported by NIH P20GM103434 and NIGMS U54GM104942)
Title: Targeted disruption of transcriptional effector GLI2 attenuates breast tumor growth and metastasis

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Body: We reported that transcriptional regulation by the major Hedgehog (HH) pathway effector GLI2 in mammary gland stromal cells coordinates a hormone-responsive niche signaling program that directs epithelial stem cell activity during puberty [Zhao, et al., Science. 2017 Apr 21;356(6335)]. HH signaling is a key growth pathway in human carcinogenesis. The first targeted therapies aimed at G-protein coupled receptor Smoothened (SMO) resulted in rapid acquired resistance, underscoring the need for developing new HH pathway-targeting therapies downstream of SMO. In BC GLI-dependent transcription may involve a non-receptor-based mechanism of GLI2 activation involving cross-talk from other signaling pathways such as TGFβ54, PI3K55,56, Wnt, or NF-κB. Accordingly, we investigated the role of HH pathway and its transcriptional effectors using the MMTV-PyMT BC mouse model. Development of mammary tumors arising proceeds through neoplastic lesions ranging from carcinoma in situ (10-12 weeks) to highly invasive ductal carcinoma with high incidence of pulmonary metastasis at 16-18 weeks. Epithelial ablation of GLI2 dramatically reduced tumor progression. Cre recombinase under control of the Ck14 promoter to genetically ablate GLI2 in basal cells of the mammary gland thus dramatically attenuated metastasis despite continued (albeit reduced) formation of primary tumors. In human BC, we applied Bayesian methods to gene expression data to identify metastatic BC with HH pathway activation. Kaplan-Meier analysis demonstrated significantly worse progression-free survival in patients with HH pathway activity (Log-rank p = 0.0013). Next, we analyzed 1294 BC samples, stratified according to HH activity, using the HH probability as a continuous score ranging between 0 and 1, univariate Cox regression analysis supports the hypothesis that HH activity in BC is a risk factor for relapse after surgery, HR = 2.45 (95% CI: 1.67 – 3.61, p = 2.72e-6). There is also a significant difference in survival between HH-actives and HH-inactives (Log-rank p = 3.91e-4), suggesting a pathogenic role of GLI activation in BC progression and metastasis. Arsenic trioxide (ATO), inhibits HH pathway activity by de-stabilizing the GLI transcriptional effectors of HH signaling, likely due to arsenic displacement of zinc within the DNA-binding zinc fingers of GLI proteins. ATO treatment of BC cell lines resulted in dose-dependent cell growth inhibition (Alamar Blue) and induction of apoptosis (PARP cleavage and Annexin V expression) at clinically achievable concentrations. In NSG mice with orthotopic transplant of 3e5 SU151 human BC PDX, with strongly positive GLI-active signature, daily IP injection of 10mg/kg ATO resulted in marked tumor growth inhibition in vivo (2.75-fold smaller tumor diameter vs. PBS vehicle controls, P= 0.006; 55d vs. 17d to reach 1.5 cm criterion for ATO vs. vehicle). We conclude the major HH pathway transcriptional effector GLI2 coordinates a transcriptional program with a central role in BC growth and metastasis in a significant subset of BC, and that systemic treatment with ATO (new oral formulation in commercial development), will reduce metastatic progression and improve clinical outcomes in patients whose tumors harbor a GLI-active transcript signature.
Title: The TRANSERI project: Effect of eribulin (E) in patients with metastatic breast cancer (mBC) on circulating TGFβ and TNFα. Relationship with outcome

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Body: Background: E is approved for the treatment of mBC patients (pts) after failure of at least 2 previous chemotherapy (CT) regimens containing antracyclines and taxanes. Its mechanism of action interferes with microtubule leading to cell cycle arrest in G2/M phase and cell apoptosis. An in vitro study in triple negative BC cell lines shows that exposure to E reverses epithelial mesenchimal transition (EMT) phenotype toward an epithelial morphology and induces changes of gene profiling and protein expression. Accordingly in mice E reduces metastatization and can reverse EMT. TGFβ is an immunosuppressive cytokine and a growth factor for cancer-associated fibroblasts (CAFs). In addition it drives EMT. TNFα synergizes with TGFβ to promote EMT. While CAFs pave the way for metastatization, EMT permits cancer cells trafficking through the blood flow following CAFs and finally developing metastases.

The purpose of the study is to investigate whether E interferes with TGFβ and TNFα levels and if the changes correlate with the outcome and the metastatic spread.

Methods: Pts with mBC, after failure of at least 2 previous CT lines were treated with E delivered at 1.23 mg/m², d 1–8 every 21 d. Blood levels of TGFβ and TNFα were determined at baseline, before cycle 3, 5 and at disease progression. The changes observed were correlated with the outcome and the metastatic spread.

Results: The study is ongoing. Here we report preliminary data on 16 pts who completed 3 cycles of E. No change of TNFα level was observed during treatment. On the contrary, TGFβ levels changed during treatment. Basal levels of TGFβ were divided in upper or lower the median (m) value. We did not observe any difference in m PFS between high or low values (137 d vs 141). However the m TGFβ value in pts was much higher than that observed in 3 healthy volunteers (m concentrations: 205 pg/ml [C.I. 115-920] vs 108 pg/ml [C.I. 85-120] respectively).

In 5 pts, TGFβ increased between cycle 1 and cycle 3, while diminished in 11 pts. We observed a numerical difference in PFS between the pts with decreased and increased values (150 d vs 85, p=NS).

We then divided the population in 3 groups: pts with TGFβ increased more than 25% of their basal levels (increased), pts with changes between +25% and -25% compared to their basal levels (stable) and pts with decreased values more than 25% of their basal levels (decreased). Comparing “increased” vs “stable” + “decreased” we observed a trend toward longer PFS favouring the latter group (77 d vs 144 p=0.12).

We collected the third determination in 14 pts. We did not analyse these data yet. However we observed that in 6 pts, TGFβ continues to decline. None of these pts progressed. In these pts the m value of TGFβ approaches healthy controls value (m concentrations: 180 pg/ml [C.I. 200-100] vs 108 pg/ml [C.I. 85-120]).

Conclusions: TNFα does not change during E treatment. On the contrary, TGFβ changes compared to basal levels. In pts with increased TGFβ between cycle 1 and 3 the PFS is lower than that observed in pts with stable or decreased levels (p=0.12). Pts with continue decline of TGFβ at cycle 5, approach the values of the healthy volunteers. None of them progressed. Updated results will be presented.
The KRAS-variant induces an EMT in normal breast epithelial cells

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Background: The KRAS-variant is a functional, microRNA binding site variant in the 3'untranslated regions (3'UTR) of the KRAS oncogene, which predicts increased cancer risk for certain populations of patients, including an increased risk of triple negative breast cancer [1], and multiple primary breast cancers [2]. Tumors in KRAS-variant patients exhibit altered biology with a KRAS-addicted signature, an estrogen negative basal-like gene expression pattern, and a unique response to cancer therapy. Our goal was to better understand the underlying normal cellular biology associated with the KRAS-variant using an isogenic normal epithelial cell line model.

Methods: Perfectly matched, isogenic normal breast epithelial cell lines with (MCF10A^{KRAS+/-}; MT1 and MT2) versus without the KRAS-variant (MCF10A^{KRAS-/-}, WT) were created and used in these studies [2]. The underlying molecular changes leading to EMT, stem cell phenotypes and altered microRNA expression were evaluated by targeted qRT-PCR, immunofluorescence, immunoblotting, immunoprecipitation, flow cytometry, global microRNA array and human phospho-kinase array analysis.

Results: We found that KRAS-variant MT cells display a mesenchymal phenotype. We confirmed the downregulation of epithelial markers (E-cadherin and Occludin), upregulation of mesenchymal markers (Vimentin, Fibronectin and N-cadherin) and transcription factors (SNAIL and ZEB1) involved in EMT, as well as decreased level of miR-200c (1000-fold), consistent with EMT induction in the presence of the KRAS-variant. We found that KRAS-variant MT cells also exhibited elevated TGFβ, consistent with our findings in KRAS-variant patients [3]. We further found that TGFβ signaling in KRAS-variant MT cells appears to lead to EMT through non-canonical signaling through MAPK (ERK) and PI3K (AKT) and AKT/GSK3β/Snail pathways.

Conclusions: KRAS-variant MT normal epithelial cells exhibit an EMT phenotype and genotype, apparently through a non-canonical TGFβ/AKT-mediated pathway. This is the first evidence to our knowledge of the powerful impact that a 3'UTR germ-line mutation can have on epithelial normal cellular biology, opening up numerous avenues for further elucidation of germ-line human variants in biology and their clinical applications to breast cancer risk.

References:
Title: Aggressiveness of epithelial cancers is independent of epithelial-to-mesenchymal transition

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Body: Background: Epithelial-to-Mesenchymal Transition (EMT) is postulated to be an important step in cancer progression and controlled by multiple mechanisms including EMT transcription factors (EMT-TFs) and splicing factors such as Epithelial Splicing Regulatory Proteins (ESRP1 and ESRP2). We previously have shown that the expression of ESRP1 and ESRP2 have significantly elevated in cases with high Oncotype DX scores and in ERα-positive cells with acquired endocrine resistance (SABCS 2013). This study seeks to identify the role of EMT-TFs and ESRPs in the determination of outcomes of patients with ER+ breast cancer.

Patients and Methods: The expression of EMT-TFs and ESRP1 was analyzed in the Affymetrix microarray and TCGA BRCA databases. Next, we generated genetically engineered knockdown models of ESRP1 to understand its functional role in endocrine resistance. We performed RNA-seq and MATS analysis to identify alternative splicing events (ASEs) between ESRP1 knockdown and control cell lines [(2C3 vs 2-control (LCC2 set) and 9C2 vs 9-control(LCC9 set)]. Validation of the ASEs was performed using a probe-based platform [Human Transcriptome Array 2.0 (HTA)] and TCGA SpliceSeq from breast tumors.

Results: High levels of ESRP1 mRNA, but not EMT-TFs, are associated with poor prognosis in human ER+ breast tumors (Affymetrix; \( P=2.8e-07 \) and TCGA; \( P=0.00011 \)). Knockdown of ESRP1 in ER+ endocrine resistant breast cancer induced glandular differentiation, rather than mesenchymal features. This was associated with significant reduction in cell and tumor growth in mammary fat pad orthotopic xenograft mice models of LCC2 and LCC9. No alterations in EMT-TFs were observed in these cells. Transcriptome profiling of ESRP1 knockdown cells further revealed altered ASEs in EMT splicing gene signature, but not at the gene level. These alterations (SE-skipped exon) were further validated for ARHGEF11, ENAH, FNIP1, SCRIB, and SLK using probe based HTA platform for ESRP1 knockdown cells and TCGA-SpliceSeq ER+ BRCA tumors in ER+ ESRP1low versus ESRP1high breast tumors.

Conclusions: Our data demonstrates for the first time that high ESRP1 is associated with poor prognosis in ER+ breast cancer. Despite its involvement in regulation of EMT splicing signature, low levels (or knockdown) of ESRP1 were not associated with EMT phenotype in tumors or in endocrine-resistant ER+ cells. Taken together, our findings indicate that EMT is not important in determining prognosis in ER+ breast cancer and that ESRP1 exerts a different role in aggressive ER+ breast cancers.
Title: Eribulin differentially disrupts TGF-β signaling pathway in BT-549 and HCC1937 breast cancer cell lines

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Body: Microtubule targeting agents (MTAs) continue to be valuable in treating breast cancer. While these drugs were classically identified as only antimitotic agents, recent evidence demonstrates that the ability of MTAs to disrupt key signaling components in interphase cells likely contributes to their anticancer actions. Yoshida and colleagues demonstrated that eribulin reverses TGF-β-induced epithelial-to-mesenchymal transition (EMT) in preclinical models of triple negative breast cancer (TNBC) within 7 days. To gain a deeper understanding of the interphase effects of eribulin in comparison with other MTAs on the TGF-β signaling pathway, we tested the hypothesis that a short-term, 2 h treatment of TNBC cells with MTAs would disrupt TGF-β signaling. The effects of the microtubule destabilizers eribulin and vinorelbine and the microtubule stabilizers paclitaxel and ixabepilone on downstream targets of the TGF-β pathway were evaluated in two TNBC cell lines. In the mesenchymal type BT-549 cells, eribulin and vinorelbine significantly inhibited TGF-β induced expression of Snail and Slug, while the microtubule stabilizers had no effect on their expression. In the basal-like 1 type HCC1937 cells, the microtubule destabilizers inhibited the expression of Snail when compared to vehicle and stabilizer-treated cells, but caused increased expression of Slug. These data suggest that MTAs have different effects on the TGF-β signaling pathway in distinct molecular contexts. Our efforts are directed towards deciphering the effects of eribulin and other MTAs on the TGF-β pathway and how this contributes to differential sensitivity. Preliminary data suggest that eribulin initiated inhibition of Snail and Slug in BT-549 cells is mediated by the scaffold protein NEDD9 and we are testing whether the differential levels of expression of NEDD9 help explain the differences in the effects of eribulin in BT-549 and HCC1937 cells. These results will begin to decipher differences among MTAs in different molecular contexts and might help identify biomarkers for optimal therapies. Funding for this work was provided by Eisai Inc.
Title: A molecularly annotated collection of breast cancer patient-derived xenograft models aligned with ongoing clinical trials built from fine needle aspiration samples throughout neoadjuvant treatment

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Body: BACKGROUND: Patient-derived xenograft (PDX) models of breast cancer replicate the diverse histologic and molecular features of patient tumors and provide a renewable source of human tumor tissue. However, collection of tissue by core needle biopsy is problematic due to patient discomfort, bleeding risk and the limited number of passes a patient can tolerate. Several studies have catalogued the maintenance of molecular features of patient tumors in PDX models of breast cancer.

METHODS: To support the neoadjuvant molecular diagnostic and drug development program in triple negative breast cancer (TNBC), a pilot study was conducted to determine if fine needle aspiration (FNA) could be used for building PDX models. Subsequently, PDX models are being established in alignment with ongoing clinical trials at MDACC. The molecular evolution of patient's tumors, matched with PDXs engrafted from their tumors, is under study throughout the neoadjuvant treatment of TNBC using RNA sequencing, whole-exome sequencing, deep sequencing of cancer genes, and histologic analyses.

RESULTS: To date, 20 established PDX models have been developed and stable PDX models continue to be generated at a rate of 2-3 per month. Several of these models are derived from serial FNAs derived from patients throughout neoadjuvant treatment. These models retain histologic and molecular features of the original patient tumors. Serial patient biopsies, matched with PDX models, have enabled measurement of the mutational and transcriptomic evolution in vivo of TNBC undergoing neoadjuvant treatment.

We have standardized the use of FNAs to generate PDX models both pre- and post-neoadjuvant therapy in the following ongoing neoadjuvant clinical trials:

1. MDACC 2014-0185 (PI Stacy Moulder, 360 patients), 'ARTEMIS: A Randomized TNBC-Enrolling trial to confirm Molecular profiling Improves Survival' 
2. MDACC 2014-0045 (PI Jennifer Litton, 20+ patients), 'A pilot study of BMN673 as a neoadjuvant study in patients with a diagnosis of invasive breast cancer and a deleterious BRCA mutation'

CONCLUSION: We demonstrated that PDX models from tissue collected by FNA recapitulate the biology and clinical course of the patient's tumor. Sequencing analyses revealed that neoadjuvant chemotherapy and PDX engraftment enrich for cancer gene mutations. We observe association of the rate of successful PDX engraftment with clinical parameters such as the patient's residual cancer burden (RCB) status at the time of surgery (upon completion of neoadjuvant treatment). In addition, we observe that PDX models derived from serial patient biopsies throughout treatment are more resistant to chemotherapy treatment. These models recapitulate the variety of chemotherapy responses observed in patients with TNBC and serve as powerful tools for preclinical biomarker and discovery studies.
2017 San Antonio Breast Cancer Symposium

Publication Number: P5-05-02

Title: A laboratory/machine learning based comparative genetics model accurately predicts breast cancer in a human cohort

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Body: Inherited genetic variants are estimated to account for 30-35% of overall breast cancer risk. A few rare, highly penetrant, genetic determinants of risk in humans have been well defined. However, the actions of many common weakly penetrant breast cancer risk loci remain uncharacterized. Additionally, it is well established that endocrine factors in general, and estrogens in particular, influence breast cancer etiology. We are using the ACI rat model of 17β-estradiol (E2)-induced mammary cancer to parse the contributions of individual genetic risk variants to breast cancer susceptibility in a physiologically relevant context. ACI females develop mammary carcinomas at an incidence approaching 100% when exposed to physiological levels of E2, and these carcinomas share many features with luminal-type breast cancers in humans. In contrast, Brown Norway (BN) rats are highly resistant to E2-induced mammary cancer. Linkage analyses of progeny from intercrosses between susceptible ACI and resistant BN rats led to the identification of multiple quantitative trait loci for E2-induced mammary cancer. One such locus, *Estrogen-induced mammary cancer 4* (*Emca4*), is the focus of the current investigation. We generated a series of novel congenic rat strains which carry BN alleles at distinct regions of interest across the *Emca4* locus, introgressed onto the ACI genetic background. Characterization of mammary cancer phenotypes in the congenic strains facilitated fine resolution mapping of the *Emca4* locus. These studies revealed that *Emca4* is a complex locus harboring at least four interacting genetic determinants of risk, designated *Emca4.1 – Emca4.4*, and is orthologous to the 8q24.21 breast cancer risk locus in humans. To assess the relevance of the rat genetic data to human populations, novel machine learning methods were employed to generate risk prediction models using data from a human cohort. Genotype data for 76 SNPs located in the regions of the human genome orthologous to *Emca4.1 – 4.4* were obtained from the Cancer Genetics Markers of Susceptibility case control population. Models generated from this data set were optimized with novel algorithms to identify a subset of 16 variants that significantly influenced the risk models. The best model distinguished breast cancer cases from controls with a remarkably high degree of accuracy for a model based on genotype (AUC = 0.6, $P < 10^{-11}$ relative to random guessing). It is worth noting that the predictive power of this model arose from interactions between human SNPs. Our data show that *Emca4* is a complex locus containing multiple interacting determinants of risk; variants in the orthologous 8q24.21 breast cancer risk locus in human interact to influence breast cancer risk as predicted by the rat model; and accounting for interactions between variants achieves a predictive power beyond what is observed with individual SNPs. We have demonstrated, for the first time, the ability to develop a multi-component genetic model in rats and test it in a human population. This illustrates the power of the rat model to elucidate the complex mechanisms through which common, weakly penetrant variants influence breast cancer risk in humans.
Title: Obesity drives breast cancer progression through estrogen dependent and independent mechanisms

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Body: Background: More than 200,000 women are diagnosed with breast cancer each year and 75% develop estrogen receptor positive (ER+) tumors. Obesity is an independent risk factor for the development of ER+ breast cancer, particularly after menopause, and affects 40% of US women. Obese women are more likely to be diagnosed with advanced tumors, lymph node involvement, and less likely to respond to endocrine therapy versus lean women. Mechanisms underlying the increased risk and worse prognosis of obese women are poorly understood. The dogma in the field states that estrogen production is the main contributor to obesity-associated ER+ breast cancer. We show that this is not always the case. Given the epidemic proportions of obesity in the US, we need better pre-clinical models that will inform focused clinical trials and interventions for patients. In this study, we describe a novel mouse model of obesity and ER+ breast cancer patient-derived xenografts (PDX). Our studies highlight the heterogeneity of responses within the ER+ breast cancer subtype to the obese environment and implicate both estrogen-dependent and independent mechanisms of obesity-associated tumor progression.

Methods: ER+, FGFR1-amplified or non-amplified human tumors were established in ovariectomized lean and obese mice in the presence of high or low estradiol (E2). To simulate the hormonal environment of women on aromatase inhibitors, E2 was removed from half of the mice in each adiposity group and the study was terminated 3 weeks later. Weight gain, body fat percentage, and adipose tissue as well as tumor characteristics were analyzed.

Results: Prior to EWD, obese mice were heavier and had higher body fat percentage than lean mice and also displayed a phenotype of metabolic dysfunction. This trend was accelerated after EWD, with obese mice gaining more weight due to body fat accumulation. Tumors responded in one of two ways: Regardless of FGFR1 amplification, obesity promoted ER+ tumor growth in the presence of low (postmenopausal), but not high (premenopausal) E2. In the presence of low E2, tumor PR levels were higher in obese compared to lean mice, suggesting hyperactive ER signaling. In FGFR1-amplified tumors, obesity promoted tumor growth after EWD. In addition, EWD induced excess fat deposition in visceral depots in both lean and obese mice; however, obese mice also gained fat in mammary adipose depots. Mammary fat pad mass and rate of post-EWD weight gain directly correlated with adipose FGF1 levels. Tumors from obese mice had higher levels of phosphorylated FGFR1, without changes in total FGFR1, compared to lean mice.

Conclusions: Utilizing a unique PDX model system, we show that obesity promotes tumor progression in the presence of low E2, and also after EWD, and identify growth factor receptor signaling as a mediator of these phenotypes. The activation of FGFR1 may underlie increased breast cancer risk and recurrence observed in obese, postmenopausal women.
Body: Background: Massively parallel DNA sequencing efforts have facilitated the production of catalogs of the somatic mutations present in cancer genomes. Examining the sequence context of the mutations it is possible to identify patterns that recur frequently. These mutational patterns or “signatures” enable inference into the mutational processes responsible for the patterns. Investigators at the Sanger Institute in the UK have produced a compendium of 30 “Signatures of Mutational Processes in Human Cancer”. Two of these signatures have been attributed to the actions of APOBEC cytidine deaminases. Data from other investigators suggest that APOBEC3B (A3B) is more likely to play a role than are other members of the APOBEC family. The evidence to support the hypothesis that APOBEC activation is responsible for the mutation patterns is limited at present. It is important to establish whether these enzymes play a role in oncogenesis as the APOBEC mutational signature had been noted to be present in 1 of every 6 cancer specimens and second only to aging in prevalence.

Methods: In order to determine if overexpression of APOBEC3B in the mammary gland of mice is tumorigenic and produces the mutational signatures attributed to its activity, we contracted with a commercial vendor for the production of an APOBEC3B transgenic mouse on a 129:C57BL/6 background. Of note, in the course of the production of the transgenic, the intron between exons 5 and 6 was retained in the construct to prevent the expression of the highly mutagentic protein in E. coli. The transgene was placed 3’ of a stop sequence flanked by LoxP sites within the ROSA26 locus. Mating with MMTV-Cre mice restricts the excision of the stop cassette and expression of APOBEC3B to the mammary tissue. A3B heterozygotes from the F1 generation were bred to produce A3B homozygotes. Homozygotes have been allowed to undergo multiple pregnancies and have been observed for up to 12 months as of June 2017.

Results: 3 mice have developed malignancies. Two mice appear to have developed lymphomas and the epithelial cells of a mammary gland of a third mouse (homozygote) at 11 months of age are diffusely hyperplastic. The cells are arranged in rows ranging from 2-4 cells in thickness. Periodic acid–Schiff (PAS) staining confirms that the basement membrane is intact around the hyperplastic cells. There is no anisocytosis, anisokaryosis, desmoplasia, necrosis or mitotic figures noted in the gland.

Conclusions: At 11 month of age, the mammary glands of APOBEC3B transgenic mice are beginning to display histologic abnormalities associated with breast oncogenesis. Observation will continue. With the passage of time we expect to observe more advanced and larger mammary lesions. DNA will be extracted from any invasive mammary tumors, sequenced and the mutation signature determined using the EMu: Expectation-Maximisation inference of mutational signatures software available from Sanger.
Body: Overall, triple negative breast cancers (TNBCs) constitute 12% of all breast cancers, and is approximately twice more prevalent in African-American populations. Louisiana has a high proportion of African-American residents (32.5% in 2015), and thus hosts a higher population of TNBC patients. TNBCs have an aggressive phenotype that is elusive to the targeted therapeutics used to treat other breast cancer subtypes. Certain kinase families have been extensively studied as regulators of epithelial-mesenchymal transition (EMT), a process involved in the initiation of cancer metastasis. Discovery of novel kinase targets within the subset of uncharacterized kinases could provide important insight into future targeted therapies. However, current models utilized in target discovery research are limited by the inability to accurately recapitulate the complex stromal architecture and heterogenous genetic and molecular composition of breast cancer. Furthermore, immortalized cell lines are limited to a 2D environment and over time acquire mutations that may not reflect the primary tumor. Recently, our laboratory has successfully established two TNBC patient-derived xenograft (PDX) models derived from African-American patients, and generated cell lines (TU-BCx-2K1, TU-BCx-200) and mammospheres. One of these models, 200, presents tumor architecture, cellular composition, genomic (qRT-PCR) and protein (western blot) expressions that are concordant with a claudin-low subtype, which has higher rates of metastasis and recurrence. Furthermore, we show that both TNBC models metastasize to the lungs, and exhibit molecular characteristics consistent with mesenchymal phenotypes. We utilized these translational PDX models to screen a library of small molecule inhibitors that represent a variety of kinase pathways to identify novel therapeutic targets and/or pathways that are specific to TNBC subtypes. We found in a preliminary cell morphology screen using three TNBC cell lines (MDA-MB-231, BT549, MDA-MB-157), two small molecule inhibitors that increased epithelial marker (CDH1) gene expression, suppressed mesenchymal (VIM, c-FOS, SNAI1, ZEB1) expression and/or suppressed cellular motility in transwell migration assays. We observed after ex vivo treatments with our PDX tumors the two compounds increase the epithelial marker CDH1 expression, and suppress mesenchymal markers (VIM, MMP2, c-FOS, SNAI1, ZEB1) expressions. We confirm these findings in the TU-BCx-2K1 cell line. Kinase array data revealed candidate kinases responsible for the observed EMT changes in the two compounds of interest (NEK5, NEK9, NEK1 potentially affect cell motility; SRC-family kinases, TAOK2, STK10 potentially affect EMT gene changes); we plan to utilize the PDX cell lines to characterize these kinases in EMT. We aim to ultimately discover novel therapeutic targets specific to different TNBC molecular subtypes.
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Title: Development of advanced pre-clinical in vivo models of metastatic breast cancer

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Body: Backgrounds: The fact that we continue to lose 40,000 women with breast cancer every year in the US despite the recent advance in basic research clearly demonstrate disconnect in translation of basic research findings to clinic. This is largely due to lack of appropriate animal model that mimic clinical conditions for preclinical studies that result in high failure rate of clinical trials. To date, we had established many syngeneic mouse models, which are not free from limitations; 1) few clinically relevant animal models with bone metastasis have been established, 2) syngeneic mouse model cannot address human cancer genomics and tumor heterogeneity. Patient-Derived Xenograft (PDX) model has emerged as pre-clinical model to address these issues, however, it suffers low tumor take rate of around 20-40%, and lack metastatic model. Here, we describe development of orthotopic implantation, and bone and liver metastatic breast cancer mouse models to overcome these limitations.

Methods: 1) 4T1.2-luc3 cells that has metastatic potential to the bone were orthotropically inoculated as a syngeneic mouse model, imaged with IVIS and MRI. 2) Patient tumor tissues of 1mm(3) were implanted surgically into dorsal subcutaneous space (SQ), or orthotropically into mammary fat pat #2 and #4 (MFP).

Results: 1) We established a syngeneic breast cancer bone metastasis model. Primary tumors were surgically resected days after 4T1.2-luc3 cells were orthotopically implanted under direct vision. Removal of primary tumor allowed bioluminescent visualization and quantification of bone metastasis by IVIS. We found that MRI was effective in evaluating bone metastasis and bone related events in these mice. MRI allows differentiation of bone metastasis from metastasis to the surrounding organs with bone destruction image, whereas conventional bioluminescence imaging shows only existence of cancer cells. 2) The overall tumor take rate of the tumor in PDX model was 46.0% (74/161 implantation site). Take rate from triple-negative breast cancer tumors was 56.1% (74/132), on the other hand, that from ER positive tumors was 0% (0/39). Tumor take rate was significantly better in MFP implantation than SQ (39.5%, 30/76 vs 51.2%, 44/85, p<0.01). Tumor weight were significantly heavier in MFP compared to SQ (0.072g vs 0.328g, p<0.00001). With more passage, the difference in tumor weight between SQ and MFP was significantly increased(p<0.0001). Finally, we developed a PDX breast cancer liver metastasis model by surgically implanting tissue fragment into liver using direct vision technique. We found MRI to be very useful as a living imaging modality to evaluate cancer progression in the deeply located metastatic sites of PDX models.

Conclusions: We have established orthotopic syngeneic breast cancer bone metastasis model as well as improved breast cancer PDX model with synchronous liver metastasis utilizing MRI. Our novel models could be powerful tools for preclinical studies.
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Publication Number: P5-06-01

Title: Proteomic analysis of conserved kinases between PDX tumors and corresponding PDX-derived cell lines

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Body: (Background) Patient-derived xenograft (PDX)s are valuable models for precision oncology as they are thought to recapitulate the biological and genomic characteristics of the human tumors they were derived from. Even though this model is widely used, it is still difficult to manipulate genes in this system and it is not as convenient as in vitro cell culture for drug sensitivity screening. On the other hand in vitro cell cultures have a highly artificial microenvironment and also have undergone selection which might generate misleading data. To address these issues cell lines from PDX tumors with different intrinsic subtypes were characterized by kinobead precipitation/mass spectrometry analysis (KiP/MS) to profile each PDX line in vivo and ex vivo for therapeutic targets.

(Materials and Methods) Washington University Human in Mice (WHIM) tumors and Hutsman Cancer Institute (HCI) tumors were transplanted into mammary fat pads of female severe combined immunodeficiency/beige (SCID/beige) mice. PDXs tumors were harvested when they reached 1~1.5cm. PDX tumor-derived ex vivo cells/organoids were routinely cultured with Rock inhibitor support. Estradiol was applied only for the tumors originating from E2 supplementation in vivo. Cells and tumors were harvested and lysed by sonication. Kinases in soluble lysates are enriched with drug-bound beads (kinobead) and digested with trypsin. Digested peptides are analyzed by mass spectrometry.

(Results) 5 of WHIM tumors and 4 of HCI tumors were successfully dissociated and cultured ex vivo for further analysis and experiments. Hierarchical clustering of tumors and corresponding cells showed ex vivo cultured cells cluster together with their original PDX tumors. Tumors/ex vivo cultured cells cluster by intrinsic subtype and enrichment analysis identified specific kinases for each PDX tumor/cell line. The WHIM 4 tumor-cell pair showed high level of PIM kinase and EGFR and other PDX tumors such as WHIM18, WHIM 20 (Luminal subtypes), WHIM 35 (HER2 enriched subtype) also showed model unique intrinsic kinases, such as JAK2 for WHIM18, EPHB4 for WHIM20.

(Conclusion) PDX tumor-derived ex vivo lines could be routinely cultured with Rock inhibitor support. Each PDX tumor and cell line pair were cluster together in hierarchical clustering and categorized into the same intrinsic subtype based on kinome profiling, suggesting these cells maintain their tumor specific intrinsic kinase signaling. A subset of kinases exhibit activity/expression that is conserved after ex vivo culture, we hypothesize these are intrinsic kinases might be promising target for treatment, because they are tumor intrinsic, i.e. their high expression is maintained despite the strong contrast in the microenvironment of in vitro versus in vitro growth. Ongoing studies with drugs and knock down reagents are examining whether the in vivo/ex vivo comparative KIP analysis indeed identifies therapeutic targets, which will be presented at the meeting more in detail.
Title: Ex vivo CSC assays for personalized testing of drug susceptibility in advanced breast cancer

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Body: In the developing area of personalized medicine, targeted therapies are mainly based on genomic characterization of each tumor, and is currently proposed as promising strategies for advanced breast cancer (ABC). Despite the promises of advanced genome sequencing, many patients still fail therapy, resulting in disease progression, recurrence, and metastases. Cancer stem cells (CSCs) concept illustrates the non-genetic intrinsic resistance, recapitulates tumor heterogeneity that creates hierarchically organized tumor tissues where a subpopulation of self-renewing cancer stem cells (CSCs) sustains the long-term clonal maintenance of the neoplasm. Evidences indicate that CSCs survive many commonly employed cancer therapeutics. Patient-derived tumor xenograft (PDXs) models recapitulate tumor complexity and heterogeneity at cellular and molecular level. We aimed to specifically address the therapeutic sensitivity in ABC, by using an ex vivo assay based on PDX prospective collection, fully characterized for genomic alterations.

In this work, we aim at defining for each tumor the best therapy to target breast cancer intratumor heterogeneity, the CSC component. For that, we defined a panel of 44 FDA-approved compounds used for cancer treatment, including breast and other types of cancer, cancer stem cell drugs, chemo or targeted therapies. For each drug, we screened the differential sensitivity of the bulk tumor cells and the CSC components for 12 PDX models using an ex vivo screening approach on short term culture. To assess intra tumor heterogeneity, we set up an original dual strategy: for the bulk cells, an ex vivo assay based on IC50, and for breast CSC component a miniaturized Aldefluor assay. First, we demonstrate that bulk cells and CSCs sensitivity may be dissociated for the same drug in the same PDX models. Then, we observed that whereas bulk cell sensitivity may be correlated to tumor genomic abnormalities, CSC drug sensitivity seems not to follow the rule. CSC are selectively sensitive to specific compounds. We are exploring the pathways that sustain this selective sensitivity in the CSCs components. We are currently identifying targets using mass spectrometry in CSCs and bulk cells. Then, we validated the hits predicted from ex vivo screening assays by in vivo treatment of using PDX models for the selected drugs, and in a patient with ABC.

In that work, we demonstrated that CSCs display different sensitivity profiles than bulk cells to the same agents, irrespective to their genomic background and are identifying the CSC specific targets. Here, we propose a new model of precision medicine based on ex vivo CSC assays for personalized testing of drug susceptibility in advanced breast cancer.
**Title:** Generation and characterization of a novel invasive lobular breast carcinoma cell line WCRC-25

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**Body:**

**Background:** Invasive Lobular Breast Carcinoma (ILC) is the second most common histologic subtype of breast cancer, comprising 10-15% of all cases. ILC is clinically and molecularly distinguished from the major subtype—Invasive Ductal Carcinoma (IDC)—by loss of E-cadherin ($CDH1$). Studies on ILC remain sparse, in part due to limited cell culture models. The Women's Cancer Research Center (WCRC) has therefore set up a combined effort of breast surgical oncologists, medical oncologists, pathologists, and cancer biologists to collect fresh tissues and establish additional ILC cell lines.

**Methods:** Tumor cells were grown using irradiated fibroblast conditioned media (CM) in hypoxic (5% O$_2$) conditions. Sanger sequencing and Droplet Digital PCR (ddPCR) were utilized to test for mutation in $CDH1$ in tumor and circulating DNA (cfDNA), using germline DNA as control. DNA copy number status in the tumor was detected using nanoString approach. Expression of E-cadherin and a series of lineage markers was elucidated using Immunoblotting (IB) and Immunofluorescence (IF). A panel of ILC (MDA-MB-134, Sum44PE, IPH-926) and IDC (MCF-7, MDA-MB-231) cell lines was included for comparison. After establishment, WCRC-25 population doubling was compared between growth in CM or Dulbecco’s Modified Eagle’s Medium (DMEM), and hypoxic (5% O$_2$) or normoxic (21% O$_2$) conditions. Growth phenotypes were characterized in 2D, Ultra Low Attachment (ULA), and soft agar.

**Results:** WCRC-25 was successfully established from the pleural effusion of a 77-year old patient with metastatic ILC. The patient had stage IV (T3N3M1) ER+/PR-/HER2- ILC; was treated with bilateral mastectomy, radiation therapy, and multiple lines of chemotherapy (FOLFOX due to initial misdiagnosis; 2 cycles carboplatin/paclitaxel; anastrazole, fulvestrant, pegylated liposomal doxorubicin, gemcitabine, exemastane, and eribulin (with denosumab for bone lesions); had metastatic lesions in stomach, bone, pleura, and pericardium; and ultimately passed away from progressive pleural effusions roughly 3 years after diagnosis. A novel nonsense mutation of $CDH1$ was observed in exon 13 (Q705*) in cell line DNA, resulting in a premature stop codon. The same mutation was confirmed in cfDNA obtained from longitudinal blood samples from the patient. Copy number analysis revealed deletion of the second $CDH1$ copy in tumor, and E-cadherin protein loss was confirmed by IB and IF. WCRC-25 cells expressed epithelial cell markers CK8/18 and EpCAM, and as expected did not express stromal marker $\alpha$SMA. ER$\alpha$ expression was very low, and not sufficient for measurable hormone response. Population doubling was significantly faster in hypoxic compared to normoxic conditions, regardless of media type, but minimal in 3D.

**Conclusions:** WCRC-25 is a novel ILC cell line, defined by $CDH1$ nonsense truncating mutation and LOH, resulting in loss of E-cadherin protein expression. Cells show favorable growth characteristics in hypoxic conditions and maintain epithelial-dominated protein expression. We are currently performing RNA seq analysis of the matched primary tumor and metastatic samples from the stomach, peritoneum/falciform ligament, pleural effusion and skin, which we will compare and contrast with that of the established WCRC-25 cell line.
Classification of molecular subtypes of triple-negative breast cancer cell lines using two models

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Body: Background: Triple-negative breast cancer (TNBC) is a heterogeneous group of tumors with collective poorer prognosis and clinical outcome than other breast cancer subtypes. Understanding and defining robust TNBC molecular subtypes is important for preclinical investigations of potential therapeutics or grant proposals. The original Vanderbilt algorithm (TNBCtype), published by Lehmann et al in 2011 (J Clin Invest, 121:2750), used an identified set of 2,188 genes to classify TNBC into six subtypes displaying unique expression and ontologies - basal-like 1 and 2 (BL1 and BL2), immunomodulatory (IM), mesenchymal (M), mesenchymal stem-like (MSL), and luminal androgen receptor (LAR) - and one unstable (UNS) subtype. In 2016, the same group proposed a refined algorithm (PLoS One 11:e0157368) that included just the BL1, BL2, M, and LAR subtypes, based on their new findings suggesting that the IM and MSL subtypes were derived from infiltrating lymphocytes and other stromal cell populations within the tumor. In order to improve the reproducibility of the TNBC subtyping panel and create a cost-effective clinical tool, a new 101-gene expression panel using the same gene expression data sets used for the original TNBCtype algorithm was proposed by Ring et al (BMC Cancer 16:143, 2016). In this study, we aimed to determine the robustness of TNBC subtyping in 28 known TNBC cell lines using both algorithms in order to identify concordant cell lines that could be considered the most suitable to be used in translational research. Methods: Publicly available gene data were used to classify 28 known TNBC cell lines using the original 2,188-gene algorithm and the reduced 101-gene model algorithm. Results: Of the 28 TNBC cell lines, 18 (64%) were concordant using both TNBC classification algorithms (Table); 5 of 6 cell lines classified as BL1 by the original TNBCtype model were concordant with the 101-gene model, as well as 6 of 8 BL2, 2 of 3 M, 2 of 4 MSL, and 3 of 3 LAR cell lines. Ten cell lines had changes in their molecular subtyping using the two different algorithms. Conclusions: We speculate that TNBC cell lines that have concordant molecular subtyping in both algorithms are the most stable for defining their molecular characteristics. Therefore, for drug development studies based on the Vanderbilt TNBC molecular subtyping, we recommend using these cell lines. We plan to correlate our findings with in vivo TNBC tumor animal models to identify the best molecularly stable cell lines to be considered for research use.

Table

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**Title:** LncRNA MIR2052HG regulates ERα level and endocrine resistance through LMTK3 by recruiting early growth response protein 1

Junmei Cairns¹, James N Ingle¹, Lois E Shepherd², Michiaki Kubo³, Matthew P Goetz¹, Richard M Weinshilboum¹, Krishna R Kalari¹ and Liewei Wang¹. ¹Mayo Clinic, Rochester, MN; ²Canadian Cancer Trials Group, Kingston, ON, Canada and ³Riken Center for Integrative Medical Science, Yokohama, Japan.

**Body:** BACKGROUND: A GWAS for the MA.27 aromatase inhibitors (AIs) adjuvant trial (4,406 controls and 252 cases) identified variant (V) SNPs in a long noncoding (Inc) RNA, MIR2052HG, that were associated with longer breast cancer free interval (HR= 0.37, P= 2.15E-07). V SNPs (MAF= 0.32 to 0.42) were associated with lower MIR2052HG and ERα expression in the presence of AIs. MIR2052HG maintained ERα both by promoting AKT/FOXO3-mediated ESR1 transcription and by limiting ubiquitin-mediated ERα degradation. (Cancer Res 76:7012-23, 2016). Our goal was to further elucidate MIR2052HG’s mechanism of action.

METHODS: RNA-Binding Protein Immunoprecipitation (RBPI) assays were performed to demonstrate that the transcription factor, early growth response protein 1 (EGR1), worked together with MIR2052HG to regulate lemur tyrosine kinase-3 (LMTK3) transcription in MCF7/AC1 and CAMA-1 cells. The location of EGR1 on the LMTK3 gene locus was mapped using chromatin immunoprecipitation (ChIP) assays. The co-localization of MIR2052HG RNA and the LMTK3 gene locus was determined using RNA-DNA dual fluorescent in situ hybridization (FISH). SNP effects were evaluated using a panel of human lymphoblastoid cell lines.

RESULTS: TCGA analysis revealed LMTK3 and MIR2052HG expression were highly correlated in ERα-positive breast cancer patients. We found that the MIR2052HG transcript was located in the LMTK3 gene locus by RNA-DNA FISH. Among all of the 12 potential LMTK3 transcription factors identified in the Encode database that were examined by RBPI, only EGR1 showed an interaction with MIR2052HG. CHIP assays confirmed EGR1 binding to the two putative EGR1 binding sites in LMTK3 gene. Depletion of MIR2052HG reduced the binding of EGR1 to the LMTK3 promoter and decreased LMTK3 expression, suggesting that it might function as a scaffold. Mechanistically, decreased LMTK3 levels further increased protein kinase C (PKC) activity and downstream AKT activity, leading to reduced ESR1 mRNA levels via increased pFOXO3. At the protein level, in MIR2052HG depleted cells, increased PKC activity increased the phosphorylation of MEK, ERK, and RSK1, leading to increased ERα phosphorylation at Ser167 and increased ERα degradation. Conversely, overexpression of LMTK3 in MIR2052HG depleted cells reversed these phenotypes. MIR2052HG regulated LMTK3 and ERα expression in a SNP-dependent fashion: the MIR2052HG V SNP, relative to wild-type (WT) genotype, increased LMTK3/ERα expression in response to androstenedione due to increased binding between EGR1 and the LMTK3 promoter in LCLs. However, AI treatment reduced this binding in MIR2052HG variant cells but increased binding in WT cells, resulting in decreased LMTK3/ERα in V cells and increased expression in WT cells.

CONCLUSIONS: Our findings support a model in which the protective MIR2052HG variant genotype regulates LMTK3 via MIR2052HG/EGR1, and LMTK3 regulates ERα stability via the PKC/MEK/ERK/RSK1 axis. This regulation may explain the effect of the MIR2052HG variant genotype on cell proliferation and response to AIs in MA.27. These findings provide new insight into the mechanism of action of MIR2052HG and suggest that LMTK3 may be a new therapeutic target in ERα-positive breast cancer patients treated with AIs.
2017 San Antonio Breast Cancer Symposium

Publication Number: P5-07-02

Title: LOL acts as a luminal breast cancer-specific IncRNA to promote tumor progression in an estrogen-independent manner

Wei Sun¹, Xiao-En Xu¹, Yi-Zhou Jiang¹ and Zhi-Ming Shao¹. ¹Fudan University Shanghai Cancer Center, Shanghai, China.

Body: Luminal breast cancer is a unique subtype that express estrogen receptor alpha (ERα), and has a sustained risk of late disease recurrence and death. While luminal breast cancer can benefit from anti-estrogen therapy, some patients still suffer from drug resistance. Indicating that some estrogen-independent signal pathways may be activated in these patients. 

Using transcriptome analysis of 21 paired luminal tumor and normal tissues, we identified 133 IncRNAs that were most differently expressed, among which, 11 IncRNAs were recorded in the Ensembl or NCBI databases. The expression levels of these 11 IncRNAs in different subtype of breast cancer were analyzed using 27 breast cancer cell lines, fresh-freeze breast cancer samples, TCGA database and MiTranscriptome database. Finally, we found that only ENST0000456526 was specifically high expressed in luminal breast cancer, and almost undetectable in other subtype breast cancer, normal tissues and other cancer types. 5’ and 3’ rapid amplification of cDNA ends (RACE) PCR identified the full-length sequence of ENST0000456526 is about 1.4kb, and we named this novel IncRNA as “IncRNA of luminal (LOL)”. LOL was mainly located in the cytoplasm and had no coding probability. Downregulation of LOL reduced tumor growth via inhibiting cell cycle G1/S phase transition and inducing apoptosis. Further investigation revealed that LOL acted as a natural sponge for let-7, with as many as 7 binding sites for let-7 family at the 3’UTR of LOL sequence. LOL can promote tumor growth via both ERα-mediating pathways and ERα independent manners by regulating cell cycle related genes such as ERα, CCND1, CDC25A, DICER, PBX1 and MYC. Blocking both ERα and LOL can further reduce tumor growth comparing to ERα blockage alone in vivo and in vitro. LOL expression was higher in tamoxifen-resistant MCF7 cells. We demonstrated that LOL is regulated by cis-acting enhancers of luminal breast cancer to maintain its high expression and that LOL has a negative feedback with ERα. BRD4 inhibitors including JQ1, OTX015 and CPI0610 can significantly decrease LOL expression in a dose dependent manner. Clinical analysis with 374 luminal breast cancer samples indicated that LOL is a poor independent prognosis factor for poor survival in luminal breast cancer.

By investigating LOL, we identified an estrogen independent way of tumor progression. LOL was spliced from enhancer RNA, which can be decreased by BRD4 inhibitors, and played important roles in tamoxifen resistance. With further understanding of LOL, the mechanisms of tumor progression in luminal breast cancer will become more distinct.
Title: Suppression of NF-κB by ERα in breast cancer is mediated by a lncRNA which serves as a favorable prognostic indicator

Herbert Yu¹, Dionyssios Katsaros², Nicoletta Biglia³, Yi Shen¹, Lenora Loo¹, Xiao Yu⁴, Hongyan Lin⁴, Yuanyuan Fu¹, Wenming Chu¹, Peiwen Fei¹, Yan Ni¹, Wei Jia¹, Xiaobei Deng⁴, Biyun Qian⁴ and Zhanwei Wang¹. ¹University of Hawaii Cancer Center; ²S. Anna Hospital, n 5 and After Azienda Ospedaliero-Universitaria; ³University of Torino School of Medicine and ⁴Shanghai Jiao Tong University.

Body: Development of resistance to endocrine therapy in breast cancer patients with ER-positive tumors is a major challenge in breast cancer treatment. Multiple mechanisms have been proposed to explain the development of endocrine resistance in ER-positive patients, one of which is the reciprocal suppression of two important signal pathways in breast cancer, ERα and NF-κB. Activation of ERα is found to suppress the action of NF-κB; blocking the activity of ERα releases its suppression on NF-κB which is known to mediate strong signals in promoting tumor progression and metastasis. How ERα exerts its inhibitory effect on NF-κB remains unknown. We discovered a long non-coding RNA (lncRNA), LINC00472, whose expression is intimately associated with clinical and pathological features of breast cancer. High expression of this lncRNA was observed more often in patients with early stage disease and low grade ER-positive tumors. Patients with high expression of LINC00472 also had favorable disease-free and overall survival. These associations were confirmed in meta-analysis using multiple clinical datasets extracted from the NCBI and TCGA databases. In vitro experiments further demonstrated that overexpression of LINC00472 in breast cancer cells could slow cell proliferation, inhibit colony formation, and reduce cell migration and invasion. Reduced tumor growth was also seen in a xenograft model injected with LINC00472-overexpressed breast cancer cells. Comparison of profiles of gene expression and metabolomics between breast cancer cells with and without LINC00472 overexpression revealed that downregulation of TNF-α signaling and superpathway of methionine, respectively. These results led us to investigate NF-κB as a target of LINC00472 action. Our experiments showed that phosphorylation of IκBα and p65 were significantly declined in LINC00472 overexpression cells. Bioinformatic analysis of the LINC00472 promoter indicated a binding site for ERα. Our reporter assay confirmed that ERα binds to the promoter of LINC00472. Overexpression of ERα in breast cancer cells could upregulate the expression of LINC00472. Upregulated LINC00472 could suppress the activation of NF-κB, and the suppression could be abolished when knocking down the expression of LINC00472. Based on these clinical observations and laboratory experiments, we speculate that LINC00472 may play an important role in the development of endocrine resistance and targeting this lncRNA may help to address the development of endocrine resistance in breast cancer treatment.
Title: Nuclear miR-133 expression is associated with breast cancer progression and survival

Ming-Feng Hou¹, Fang-Ming Chen², Sheau-Fang Yang³, Hong-Ying Dai⁴ and Yao-Tsung Yeh⁵. ¹College of Medicine, Kaohsiung Medical University, and Kaohsiung Municipal Hsiao Kang Hospital, Kaohsiung, Taiwan; ²Kaohsiung Municipal Ta-Tung Hospital, Kaohsiung, Taiwan; ³Faculty of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; ⁴Institute of Biomedical Science, National Sun Yat-Sen University, Kaohsiung, Taiwan and ⁵Aging and Disease Prevention Research Center, Fooyin University, Kaohsiung, Taiwan.

Body: Background: miRNAs (microRNAs) can function as the tumor suppressor gene or oncogene to affect carcinogenesis, therapy response and progression in human cancer. The prevailing view is that miRNAs function to regulate mRNA stability and translation in the cytoplasm. However, multiple studies have detected miRNAs in the nuclear compartment. Nuclear miRNAs have been proven to regulate the functions of nuclear protein and synthesis of nuclear miRNA. We have found circulating muscle-enriched miR-133a can serve as prognostic biomarkers for breast cancer. Herein, we aim to explore the subcellular distribution, prognostic significance and treatment response of miR-133a in Taiwanese breast cancer.

Methods: Circulating miR-133a levels were conducted with the serum from 188 patients prior to surgery and from 82 healthy controls. The subcellular distribution patterns of miR-133a were analyzed by Chromogenic In Situ Hybridization (CISH) in paraffin embedded tissue sections from 224 cases. Cell viability and cell cycle were determined by XTT assay and flowcytometry. Protein and mRNA levels were examined by western blotting and RT-qPCR (TaqMan) assay.

Results: Our results showed that circulating miR-133a levels of breast cancer patient are significantly higher than those of health subjects, suggesting that increased miR-133a secretion from unknown tissues or cells may promote carcinogens in Taiwanese breast cancer. Using the receiver operating characteristic (ROC) curve to examine the diagnostic value of circulating miR-133a in breast cancer (AUC=0.834), we found that increased miR-133a levels could serve as a diagnostic biomarker in accessing Taiwanese breast cancer risk. Nevertheless, CISH analysis revealed that nuclear miR-133a frequency was inversely correlated with tumor grade, stage and lymph node metastasis (p=0.029, 0.012, and 0.0008), and cytoplasmic miR-133a intensity was positively correlated with recurrence (p=0.010). Furthermore, high frequency of nuclear miR-133a was correlated with a better overall survival rate (p=0.039) and improved the survival rate of patients who received hormone therapy (p=0.009). To further investigate the role of nuclear miR-133a, an additional miR-133a, denoted as Nu-miR-133a, ending in nuclear localization element was constructed and found to be highly enriched in the nucleus. Ectopic expression of wild-type and Nu-miR-133 inhibited proliferation of breast cancer cells. Both wild type miR-133a and Nu-miR-133a increased S phase arrest as well as the levels and foci of γ-H2AX. Unexpectedly, we found that wild type miR-133a decreased ATM expression but activated p38 MAPK. Notably, H2AX can be also phosphorylated by p38 MAPK kinase in addition to ATM and ATR. The underlying mechanism between nuclear miR-133a and potentially p38-mediated DNA damage response (DDR, γ-H2AX and S phase arrest) will be needed further elucidation.

Conclusions: In conclusion, we provide the first evidence that nuclear miR-133a may play a crucial role in the development and treatment response of breast cancer potentially through its mediated DDR. The interplay among nuclear miR-133a, p38 and DDR require further investigations.
Title: LOC283299 suppress the lymphnode metastatic cascade in breast cancer patients

Rong Guo\textsuperscript{1,2}, Jingyan Xue\textsuperscript{1,2}, Yonghui Su\textsuperscript{1,2}, Bingqiu Xiu\textsuperscript{1,2}, Weiru Ji\textsuperscript{1,2}, Yayun Chi\textsuperscript{1,2} and Jiong Wu\textsuperscript{1,2,3}. \textsuperscript{1}Fudan University Shanghai Cancer Center, Shanghai, China; \textsuperscript{2}Shanghai Medical College, Fudan University and \textsuperscript{3}Collaborative Innovation Center for Cancer Medicine.

Body: Background
Long non-coding RNAs (lncRNAs) have been proved to play an essential role in cancer metastasis. The accuracy of intraoperative assessment of lymph nodes metastasis need to be improved. In this study, we aimed to define the lncRNA biomarker ectopic expressed in breast cancer patients' metastatic lymph nodes, and to explore the potential molecular mechanisms.

Experimental Design
RNA-seq analyses in 3 paired breast cancer patients' primary tumor and metastatic lymph node was used as training set to determine differentially expressed lncRNAs that may be associated with lymph node metastasis. The other 40 patients were analyzed as validation set to test the accuracy of lncRNAs identification by quantitative real-time PCR. The correlation between LOC283299 expression level and prognosis in other 282 breast cancer patients was confirmed. In parallel, in vitro and in vivo analyses were carried out to determine the potential mechanisms of LncRNA-dependent lymph node metastases and prognosis.

Results
RNA-seq analyses in the training set revealed significant correlation between high expression level of LOC283299 and lower lymph node metastasis potential in breast cancer patients. We further validated that the expression level of LOC283299 was significantly higher in tumor primary tissue than that in paired metastatic lymph node ($P=0.0245$), and higher LOC283299 expression level was markedly associated with good metastasis-free survival in breast cancer patients ($P=0.04$). In breast cancer cell lines, CRISPR-on overexpression of LOC283299 inhibited proliferation, migration, invasion, and metastases both in vivo and in vitro. shRNA knockdown LOC283299 promotes the ability of tumor proliferation and metastasis. The molecular mechanisms by which LOC283299 as metastasis-suppressing lncRNAs regulates recurrence and metastasis may involve regulation of epithelial to mesenchymal transition (EMT), cellular invasion and microenvironment in breast cancer cells.

Conclusion
Our study revealed a strong correlation between LOC283299 expression and lymph node metastases in breast cancers. High level of LOC283299 was associated with better metastasis-free survival. The changed metastasis phenotype may be mediated by the interaction of LOC283299 and breast cancer cells. Therefore, LOC283299 may represent a potential predictive biomarker for early lymph node metastasis in breast cancer.
Title: Kaiso regulates miRNA-31 and miRNA-200 expression in triple negative breast cancer (TNBC) cells

Body: Breast cancer (BC) is the most frequent female cancer and a leading cause of female deaths worldwide. BC-related mortality rates are high among African American (AA) women despite the low incidence rates of breast cancer observed in this population compared with Caucasian Americans (CA). The triple negative breast cancer (TNBC) subtype lacks expression of three biomarkers used to clinically classify BC, and thus TNBCs cannot be treated with traditional receptor therapies. Moreover, as TNBC is biologically aggressive and women diagnosed with TNBC have poor outcomes. Interestingly, TNBC is most prevalent in young women of African Ancestry (WAA) compared to women of other ethnicities, but the cause of this racial disparity remains unknown. Recent studies in our lab revealed that the transcription factor Kaiso is highly expressed in TNBC tissues of WAA patients compared with those from Caucasian patients, suggesting a role for Kaiso in TNBC racial disparity. Intriguingly, our lab and others have also reported a correlation between high Kaiso expression, poor overall survival of AA BC patients compared with Caucasian patients, and increased TNBC aggressiveness/metastasis that is in part mediated via the TGFβ signaling pathway. Notably, Kaiso has also been implicated in tumor cell migration via its regulation of the tumor-suppressing microRNA-31 (miR-31) in prostate cancer cells. Remarkably, the pleiotropic miR-31 functions to suppress metastasis and its expression has been shown to be inversely correlated with aggressive breast tumor metastasis. Although Kaiso has been implicated in epithelial-to-mesenchymal transition (EMT) and TNBC metastasis, Kaiso's exact roles in the regulation of miRNAs in the context of TNBC remains to be elucidated.

Using chromatin immunoprecipitation (CHIP) analysis, we found that Kaiso binds to the miR-31 and miR-200 promoters, and we detected increased expression of these microRNAs in Kaiso-depleted TNBC cells using qRT-PCR analysis. Furthermore, using immunoblot analysis, we found that Kaiso depletion resulted in reduced expression of the actin remodelling protein WAVE3, which is a downstream target of both miR-31 and miR-200. Consistent with these molecular changes, transfection of TNBC cells with miR-31 and miR-200 mimics resulted in reduced migration of these cells compared to control TNBC cells as assessed via migration assays. These data suggest that Kaiso regulates miR-31 and miR-200 in TNBC cells, and promotes TNBC cell migration via downregulation of these miRNAs. Ongoing studies seek to assess and correlate miR-31 and miR-200 expression with Kaiso expression in TNBC tissues of WAA. Together, our findings raise the exciting possibility that Kaiso may be developed as a potential target for the treatment of TNBC patients.
2017 San Antonio Breast Cancer Symposium

Publication Number: P5-07-07

Title: Prognostic relevance of microRNA-155 and microRNA-21 in breast cancer patients

Sara Y Kim¹, Tsutomu Kawaguchi¹, Li Yan¹, Jessica Young¹, Qianya Qi¹ and Kazuaki Takabe¹. ¹Roswell Park Cancer Institute, Buffalo, NY.

Body: Introduction
MicroRNAs (miRNAs) are short noncoding RNA sequences that degrade or prevent the translation of their target messenger RNA (mRNA). Altered regulation of miRNAs is implicated in different cellular processes. Some miRNAs, such as miRNA-155 (miR155) and miRNA-21 (miR21), are implicated in both immunity and cancer progression. Previous studies show that both miR155 and -21 are oncogenic, as their overexpression promotes invasion, proliferation and migration of breast cancer cells in vitro. Their overexpression within patient cohorts (n= 40-173 patients) reveals a worse prognosis for miR21 and varying associations with prognosis for miR155. By using the Cancer Genome Atlas (TCGA), which contains data from over a thousand patients, we want to clarify whether high expression of miR155 or -21 is associated with an improved or worse survival within breast tumor samples. Because both miR155 and -21 are described as oncogenic, we hypothesize that high expression of these miRNAs would portend a worse survival.

Methods
Within the breast cohort, 1052/1097 patients within TCGA contained both clinical and miRNA sequence data, acquired via the Genomic Data Common (GDC) data portal. The patients were separated into a high and low expression group for both miR155 and miR21, and associations with overall survival were obtained using the Cox proportional hazard model. Furthermore, a sub-analysis was conducted based on estrogen, progesterone and Her-2 receptor status (ER, PR, Her-2) as well as TNM staging (AJCC 7th edition).

Results
General patient characteristics within the breast cancer cohort of TCGA included: 70% Caucasian, 73% >50 years old, 75% with TNM stage I and II breast cancers, 74% ER positive, and 33% Her-2 positive. We unexpectedly found that miR155 and miR21 high expression was associated with an improved survival (p=0.05 and 0.038 respectively). In the sub-analysis, a positive association with survival was seen for miR155 high expression in ER negative, and Stage I-II breast cancers (p=0.025, 0.0013 respectively), but not in Stage III-IV. The sub-analysis for miR21 found an association with improved survival for miR21 high expression in ER negative, and stage I-II patients (p=0.033, 0.0015 respectively), but not in Stage III-IV. Although not statistically significant, a trend towards improved survival was found in ER and PR positive subgroups, for both miR155 and -21. For the Her-2 negative subgroup, there was a trend for improved survival in miR155 high expression, but not in miR21 high expression. Knowing that ER negative tumors can attract more immune cells, and that miR155 and -21 can be expressed in immune cells and tumor associated fibroblasts respectively, we speculate that their high expression was concentrated within cells from the tumor microenvironment rather than the cancer cells.

Conclusion
Using TCGA as a large validation cohort, we found that high expression of miR155 and miR21 was associated with an improved survival, which was contrary to what we predicted. Future experiments using computational biology to determine the cell type composition within the TCGA tumor samples will be performed in an effort to determine whether the tumor microenvironment influenced the survival patterns we observed in the high expression groups of miR155 and -21.
Title: Survival relevance of tamoxifen sensitivity-related microRNAs, miR-342 and miR-221/222, in breast cancer patients

Jessica S Young¹, Tsutomu Kawaguchi¹, Li Yan¹, Qianya Qi¹, Song Liu¹ and Kazuaki Takabe¹. ¹Roswell Park Cancer Institute, Buffalo, NY.

Body: BACKGROUND: MicroRNAs (miRNAs) are small noncoding RNAs, which regulate the expression of target genes post-transcriptionally by RNA interference. They have emerged as one of the crucial regulators of cancer progression. Some miRNAs are reported to be related to the response of breast cancer to tamoxifen (TAM). In this study, we investigated whether the levels of TAM-resistant miRNA (miR-221/222) and TAM-sensitive miRNA (miR-342) translate to breast cancer patient survival, using multiple large databases.

MATERIALS AND METHODS: The Cancer Genome Atlas (TCGA; n=1049), Gene Expression Omnibus (GEO; GSE19536 n=96, GSE22220 n=210), and Molecular Taxonomy of Breast Cancer International Consortium (METABRIC; n=2509) datasets were used and Gene Set Enrichment Analysis (GSEA) was performed.

RESULTS: MiR-342 was identified as a TAM-sensitive miRNA, and miR-221/222 were identified as TAM-resistant miRNAs by literature search. Patients with high expression of miR-342 were shown to have better survival in TCGA (OS, p=0.02; DFS, p=0.03, respectively) and in two other independent GEO cohorts (OS, p=0.02 and p=0.0007, respectively) as well as in the METABRIC cohort (OS, miR-342-3p, p=0.006; miR-342-5p, p=0.00009). By subtype analyses, high expression of miR-342 was significantly associated with better survival in ER-positive patients (p=0.04), but not in ER-negative or triple negative patients in the TCGA cohort. This association was not observed in the METABRIC cohort. Within TCGA cohort, expression of TAM-resistant miR-221/222 did not significantly impact survival. Unexpectedly, increased expression of miR-221 was shown to have increased overall survival in all patients (p=0.00904) as well as in ER-negative patients (p=0.0479) and non-triple negative patients (p=0.0106) within the METABRIC cohort. On the other hand, low expression of miR-222 was associated with increased survival of all patients (p=0.00802) as well as in non-triple negative patients (p=0.041). Lastly, GSEA demonstrated that lower miR-342 expression was significantly seen in TAM-resistant gene sets, and higher miR-342 expression was seen TAM-sensitive gene sets, but miR-221/222 did not show any significant enrichment with TAM-resistant or TAM-sensitive gene sets. Taken together with survival data, expression levels of miR-342 reflect its TAM-sensitivity related function, however, that of miR-221/222 reflect other functions in breast cancer patients.

CONCLUSION: For the first time, we used “big data” from the TCGA, GEO and METABRIC cohorts to analyze multiple miRNAs with respect to TAM sensitivities and its survival impact. We demonstrated that expression of miR-342 reflected the sensitivity of the cancer cells to TAM sensitivity, however, that of miR-221/222 reflected other functions in breast cancer patients.
Title: Identification of miRNA biomarkers for early diagnosis of basal-like breast cancer

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Body: The routine screening for breast cancer relies primarily on imaging techniques such as mammography and ultrasonography, but it is often not sensitive enough for early detection and requires complementary approaches. Recent studies suggest that microRNAs (miRNAs) could be used as biomarkers for breast cancer due to its relative stability in archived formalin-fixed tissues as well as in bodily fluids. However, the identity of unique miRNAs that could discriminate proliferative and malignant states of the disease has remained elusive. Since TGFβ-induced epithelial-mesenchymal transition (EMT) represents a critical step during tumor progression and intravasation, studying the cancer cell at the mesenchymal state and its precursor could help to identify these critical diagnostic biomarkers. To identify novel biomarkers for early detection, we performed miRNA gene expression profiling of an established basal-like breast cancer (BLBC) model system with and without disruption of TGFβ signaling. We have identified a panel of miRNAs, including miR-210-3p, that are highly expressed in the motile, mesenchymal stage, which could potentially be used as markers in liquid biopsies for early detection of invasive BLBC. Conversely, we also found some miRNAs, including miR-200c and miR-4417, highly expressed in the noninvasive, epithelial stage. Transient overexpression of miR-4417 in mesenchymal BLBC cells was able to suppress migration and mammosphere formation in vitro, suggesting that it could block progression of BLBC, a disease highly prevalent in young African-American women and lacks targeted therapy.
Circulating microRNAs as early predictors of relapse in operable breast cancer

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**Body:** Background: Metastasis remains a major threat for patients (pts) with operable breast cancer (BC). Recurrence could arise from a state of tumour dormancy during which there is growth restriction of undetectable micrometastases. The expression of dormancy and metastasis related miRNAs was evaluated in the plasma of pts with operable BC obtained before adjuvant therapy in order to discover novel biomarkers for the prediction of relapse.

**Methods:** Plasma miR-21, miR-23b, miR-190, miR-200b and miR-200c expression was assessed by qRT-PCR in 133 pts with early BC (non-relapsed, n=84; relapsed, n=49). Expression was classified as high or low according to the median values and was associated with pts’ clinicopathological characteristics and clinical outcome.

**Results:** No correlation was observed between the expression of miRNAs and the clinicopathological characteristics of pts. After a median f-up of 90.5 mo, median Disease Free Interval (DFI) was significantly lower in pts with high compared to low miR-21 [105 mo vs not reached (NR); \(p=0.001\)], miR-200c (105.2 mo vs NR; \(p=0.007\)), or both miR-21 and miR-200c expression (81.37 mo vs NR; \(p=0.001\)). miR-21-high was also associated with decreased median Overall Survival (OS; \(p=0.041\)). In multivariate analysis the number of infiltrated axillary lymph nodes (N3 vs N0-N2, HR: 2.86; \(p=0.004\)) and miR-21 expression (high vs low, HR: 2.824; \(p=0.001\)) were independent negative prognostic factors for DFI, whereas negative hormone receptor status (HR: 3.062; \(p=0.024\)) and miR-21 high (HR: 3.545; \(p=0.029\)) independently predicted for worse OS. Moreover, miR-21 expression was higher in pts presenting early relapse (defined as relapse at \(\leq 3\) yrs) compared to those without relapse at 5 yrs (\(p=0.032\)). Furthermore, higher miR-21 (\(p=0.038\)), miR-23b (\(p=0.039\)), miR-200b (\(p=0.027\)) and miR-200c (\(p<0.001\)) levels were observed in pts with late relapse (at \(\geq 5\) yrs) compared to those without relapse.

**Conclusions:** Differential expression levels of metastasis and/or dormancy related circulating miRNAs are encountered before adjuvant therapy in pts with operable BC presenting subsequent relapse compared to non-relapsed pts. In addition, circulating miRNAs could predict for early or late recurrence years before clinical detection of metastases. These results merit prospective validation in an independent pt cohort.
Title: BHLHE40-AS1 is an enhancer associated noncoding RNA critical to breast cancer progression

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Body: Increased emphasis on breast cancer screening has led to a dramatic increase in diagnosis of ductal carcinoma in situ (DCIS). DCIS lesions are nonobligate precursors of invasive ductal carcinoma (IDC) and thus, the current standard-of-care is aggressive therapy to prevent invasive and metastatic disease. However, only ~40% of DCIS cases are predicted to progress leading to a current state of overtreatment and overdiagnosis. Thus, there is a critical need to identify functional determinants of progression of DCIS to IDC to allow discrimination between indolent and aggressive breast cancers and refine patient treatment strategies. We propose that long noncoding RNAs (lncRNAs) functionally drive breast cancer progression and their expression can discriminate between innocuous and potentially invasive DCIS.

Using biopsies from women with tandem DCIS and IDC lesions, we identified the lncRNA BHLHE40-AS1 as enriched in patient IDC. Furthermore, BHLHE40-AS1 is enriched in multiple breast cancer progression models, in HER2+ cell lines, and can be induced by expression of HER2. BHLHE40-AS1 is transcribed from a known super-enhancer that we find becomes rewired in breast cancer progression. In addition, the lncRNA is found antisense to BHLHE40, a transcription factor critical to many core processes (apoptosis, EMT, circadian rhythm). Depletion of the lncRNA attenuates expression of BHLHE40. Future studies are focused on mechanistically elaborating its function, its impact on the associated enhancer and chromatin looping, and determining the utility of BHLHE40-AS1 as a clinically relevant biomarker and therapeutic target in invasive breast cancer.
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Title: miR-34a as a prognostic biomarker for overall survival triple negative breast cancer within Tunisian patients

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Body: Introduction: miR-34a has been recently introduced to clinical trials especially in breast cancer depending on its crucial role as microRNA suppressor tumors since it regulates several oncogenes. We explore here the prognostic effect of miR-34a on triple negative breast cancer (TNBC) overall survival compared to no triple negative (NTNBC) group and its effect on PARP-1 and BRCA1 protein expression.

Methods: 76 patients with TNBC and NTNBC were recruited for this study. miR-34a was quantified using qPCR Syber Green technique. PARP-1 and BRCA1 were analyzed by IHC using Tissue Micro-Array method.

Results: PARP-1 is more expressed in TNBC compared to NTNBC; \( P = 0.04 \); RR= 1.8 (1.1-3.1), however, BRCA1 is less expressed in TNBC; \( P = 0.03 \); RR= 2 (1.2-12.6). Furthermore, we evaluated the relationship between miR-34a and clinicopathological features with survival rate of both studied groups, we found that miR-34a is more expressed among patients with distance metastases in TNBC group \( P = 0.001 \); RR= 6.5 (2.1-19.6). Survival analysis showed that miR-34a low expression in TNBC was remarkably related to overall survival; \( P=0.012 \).

Furthermore, multivariate survival test suggested that the absence of miR-34a was linked to the poor survival within TNBC according to the presence of distance metastasis; \( P=0.048 \); HR= 2.69. Our results showed that miR-34a is decreased in triple negative breast cancer patients with a very poor prognosis.

Conclusion: Our results give mechanistic insights in miR-34a as a prognostic biomarker for overall survival Triple Negative Breast Cancer within Tunisian patients through its effect on PARP-1 and BRCA1 protein expression which may open potential new strategies towards prevention and therapeutic inhibition of PARP-1 positive within mammary tumors.
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Publication Number: P5-07-13

Title: MicroRNA-mediated restoration of tamoxifen sensitivity in triple negative breast cancer through modulation of estrogen receptor alpha/beta ratio using Myrothamnus flabellifolius

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Body: Triple negative breast cancer (TNBC) represents approximately 20% of all breast cancer cases worldwide. Prognosis for this subtype of cancer is especially dire due to the absence of Estrogen (ER), Progesterone and Human Epidermal Growth Factor receptors, usually targeted by neoadjuvant therapies like Tamoxifen. Myrothamnus flabellifolius (MF), a well-studied South African medicinal herb, has shown significant potency against TNBC with minimal effect on normal cells. In this study, we use miRNA profiling, Ingenuity Pathway Analysis (Qiagen), MTT, immunoblots and immunofluorescence to characterize the targeted cytotoxic mechanism of MF in TNBC. We found deregulation in the expression of several oncogenic and anti-cancer miRNAs in TNBC post MF treatment. Using Ingenuity Pathway Analysis to analyze the miRNA expression profiles of the treated cells, we determined Estrogen Signaling as a pathway significantly affected by MF in TNBC. Immunoblots confirmed increase of the ERa/ERß ratio in TNBC, a well-studied marker for Tamoxifen-therapy sensitivity in TNBC. MTT cell viability assay and Annexin V staining confirmed increased sensitivity to Tamoxifen post treatment with low doses of MF, with up to 70% cell death at half the IC50 of Tamoxifen in TNBC. This study establishes a potential non-toxic therapeutic to supplement Estrogen-receptor targeting therapies in TNBC by increasing the ERa/ERß ratio. The data also evaluates the ERa/ERß ratio as a potential target for combination therapies in TNBC.
Title: miR-17-92 cluster, an oncogenic microRNA cluster acts as a context dependent tumour suppressor in breast cancer

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Body: Background: The miR-17-92 is an oncogenic miRNA cluster that generate six mature miRNAs: miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1, and miR-92-1. Accumulating evidences indicate the oncogenic role of the miR-17-92 cluster in human cancers. Amplification of 13q31-q32, which is the locus of the miR-17-92 cluster, have been reported in haematopoietic malignancies, such as B cell lymphoma. In contrast, MIR17HG was deleted in 21.9% of breast cancers and loss of heterozygosity at 13q12-q13 was associated with poor prognosis in breast cancer. This oncogenic miRNA cluster is deferentially expressed in various cancer. Since, miR-17-92 cluster shows differential expression among the cancers types, it is hypothesized that biological function may also vary depending on the context. Indeed, this cluster has been shown to act a tumour suppressor in some cancers. However, the functional role of this cluster in the subtypes of breast cancer remains largely unknown.

Methods: The oncomine datasets were analysed for expression of MIR17HG in breast cancer at parameters p-value threshold of 0.01 with minimum 2-fold change. We generated stable sub-clones of MCF7, T47D, SKBR3 and MDA-MB231 cells overexpressing miR-17-92 cluster by transducing with lentivirus expressing miR-17-92. Proliferation was assessed by MTS assay and colony forming assay. Cell migration was tested using scratch method. Invasion potentiality was monitored by using matrigel Boyden chamber invasion assay. For drug response analysis, control and microRNA overexpressing sub-clones were exposed to different chemotherapeutic agents at different concentrations followed by MTS assay at different time intervals. Association of miRNAs belonging to miR-17-92 with outcome in breast cancer was determined by Kaplan-Meier analysis on METABRIC dataset.

Results: We observed that expression of MIR17HG was increased in tissues and cell lines from triple negative breast cancer (TNBC) but decreased in the tissues and cell lines from the estrogen receptor (ER)-positive breast cancer. Our results showed that ectopic expression of miR-17-92 cluster significantly suppressed cell proliferation, colony formation, migration and invasion of ER-positive (MCF7, T47D) and HER2-enriched (SKBR3) cells whereas it enhanced cell proliferation, colony formation, migration and invasion in (TNBC) MDA-MB 231 cells. Further, we found that expression of miR-17-92 cluster sensitized MCF7 cells whereas it rendered SKBR3 cells resistant to chemotherapeutic compounds. Higher expression of five miRNAs of this cluster was associated with better relapse free survival (RFS) in (miR-17, miR-19a, miR-20a, miR-19b and miR-92) Luminal A subtype whereas three miRNAs of this cluster were associated with poor RFS in (miR-17, miR-18a and miR-92) HER2-enriched and (miR-17, miR-19b and miR-92) TNBC subtypes.

Conclusions: Taken together our results suggest that miR-17-92 cluster acts as a tumour suppressor in ER-positive and HER2-enriched breast cancer cells but shows oncogenic role in TNBC. Our observations underscore the functional complexity of miR-17-92 in a context-dependent and cell type-dependent manner, and more investigations are warranted to fully explore the functional complexity of miR-17-92 in subtypes of breast cancer.
Reconstructing the evolutionary paths of BIG 1-98

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**Body:** We are reconstructing the dynamics of tumour growth, treatment response, and origins of resistance of the Breast International Group (BIG) 1-98 study to determine the evolutionary paths of non-responders. We established the somatic alteration landscape of primary tumours collected at surgery from BIG 1-98 patients using targeted DNA sequencing of 287 genes of FFPE samples from 538 patients. Combined analysis of genetic and clinical data indicates increased genomic instability; TP53 mutations and copy number variations of 11q13 and 8p11 are associated with poor prognosis. We developed an *in silico* simulation framework to investigate if the non-responder outcome is due to (over)-treatment or intrinsic to evolutionary selection prior to diagnosis.

To reconstruct the evolutionary history of tumours, we created a computational multitype branching process that tracks the expansion of diverse clonal lineages as they acquire driver and passenger mutations that alter their proliferation and mutation rates. To account for the heterogeneity between patients, we created a fitting procedure based on the Cramer-von Misses statistic to find the likelihood of parameters of our computational model that recreate the mutational landscape and clinicopathological factors observed in each patient. Once the fitting procedure is done, we simulate the course of treatment following the study arm scheme accounting for the cell-cycle action mechanism of Tamoxifen and Letrozole.

Using our tool, we are characterising the range of tumour development scenarios covering different degrees of aggressiveness and genomic instability. Our computational model allows simulation of diverse adjuvant-schemes to predict optimised treatment regimes for validation in patient-derived tumour xenograft mouse models.
Identify key regulators to modulate chemo-sensitivity of triple negative breast cancer by integrative analysis

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Body: Triple negative breast cancer (TNBC), which is characterized by lack of expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor type 2 (HER2), is frequently associated with aggressive behavior and adverse patient outcomes and comprises a heterogeneous subgroup of tumors of diverse genomic features. Currently, TNBC patients are managed with only a few approved therapies and treatment strategies, including taxane-anthracycline chemotherapy. However, there is a subgroup of women with TNBC whose tumors are extremely sensitive to chemotherapy, but there are many women for whom chemotherapy is of uncertain benefit (1). To identify candidate therapeutic treatments for chemo-resistant patients of TNBC, we combined TNBC causal network modeling and chemo-response associated genes. We detected the differentially expressed genes according to chemo-responses by investigating expression profiles of TNBC samples with annotated information about responses to neoadjuvant chemotherapy (2), and constructed TNBC causal molecular network modeling by integrating epigenetic data with genetic, genomic, and transcriptomic data available for the Cancer Genome Atlas (TCGA) data (3). We leveraged the global causal network with genes associated with chemo-responses and detected highly connected chemo-sensitive subnetwork, which are enriched for Extracellular Matrix related pathways. The chemo-sensitive subnetwork was used to identify potential treatments to enhance chemosensitivity in two ways: 1) to identify key drivers that perturb genes within subnetwork based on number of directed connections, and 2) to identify repurposed therapeutics by comparing our chemo-sensitive subnetwork to the transcriptomic response to drug treatment (4). We identified the activity of microRNA let-7g was the most upstream key regulator that perturbs several collagen genes, suggesting the potential mechanism of chemosensitivity involved the extracellular matrix pathway. Additionally, we predicted candidate drugs including Genistein to modulate chemo-sensitivity, which was experimentally validated in TNBC cell lines. Taken together, the results demonstrated that our novel integrative approach of multi-omics data is useful to identify potential therapeutic targets to treat chemo-resistant TNBC patients by modulating chemo-sensitivity.

Title: Poly-ligand profiling and target identification from formalin-fixed-paraffin embedded HER2+ breast cancer specimens

Body: We have previously described the ADAPT Biotargeting System™ as a novel platform for highly multiplexed poly-ligand profiling of complex phenotypes such as drug response. Here we report extended capabilities of the this platform for target identification directly from formalin-fixed-paraffin embedded (FFPE) tissues using aptamer libraries enriched toward HER2+ breast cancer. Standard mass spectrometry-based biomarker and drug target discovery from FFPE tissues can be challenging due to limited amounts of tissue, harsh conditions of fixation and extraction and the general problem of masking by highly abundant proteins. A single stranded-oligodeoxynucleotide aptamer library was enriched on HER2+ FFPE breast cancer specimens and conjugated with biotin as well as a label transfer reagent, Sulfo-NHS-SS-Diazirine (Sulfo-SDAD). The biotinylated-SDAD conjugated library (B-SDAD-EL) was applied to HER2+ FFPE tissues and photocrosslinked to cognate binding partners within the FFPE sample in order to preserve aptamer-protein interactions under harsh denaturing conditions required for protein extraction and sample preparation. Aptamer-protein complexes were affinity purified and the label was transferred from bound aptamers to their binding partners under reducing conditions that enable proteomic digestion and high resolution mass spectrometry detection. An open database search was performed where the precursor ion tolerance was set to ± 500 Da for database searching, which enabled identification of peptides containing the transferred label as well as additional unknown variable modifications induced by the tissue fixation process. We identified proteins with known roles in HER2+ breast cancer along with several potentially drugable targets not previously associated with HER2 positivity. Differential expression of candidate targets was orthogonally confirmed by immunohistochemistry. By nature of its extreme molecular complexity and its ability to be enriched or “trained,” toward phenotypes of interest, the ADAPT Biotargeting System™ can be deployed to advance precision medicine by identifying predictive biomarkers and drug targets with novel associations to complex interactomes.
2017 San Antonio Breast Cancer Symposium

Publication Number: P5-09-01

Title: Multi-targeting of PI3K-AKT/MAPK/FAK signaling by miR-204 inhibits vasculogenic mimicry in breast cancer cells

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Body: Vascular mimicry (VM) is a novel angiogenesis-independent mechanism in which highly aggressive and metastatic epithelial tumor cells form vascular 3D channel-like structures to obtain nutrients without the use of classical blood vessels. VM is associated with poor prognosis and low survival in diverse types of human cancers. Previously, we showed that tumor suppressor miR-204 is a novel angiomiR with a pivotal role in tumor angiogenesis; however its function in VM remains unexplored. Here, we showed that metastatic triple negative breast cancer cells effectively undergo VM in hypoxia conditions. Moreover, in vitro restoration of miR-204 using RNA mimics dramatically inhibits VM. In order to decipher the signaling circuitry involved in VM inhibition by miR-204, we screened the expression levels and phosphorylation status of multiple signaling proteins using Phospho Antibody Arrays. Data showed that a signaling network of key proteins involved in the activation of PI3K-AKT, RAF1, MAPK, and FAK pathways were significantly downregulated after restoration of miR-204. In addition, a decrease in the phosphorylation of AKT (Ser473), MEK1 (Ser218/222), p38MAPK (Thr180/Tyr182), PI3K (Tyr458) and Src (Tyr416) was confirmed by Western blot. Congruently, targeted inhibition of PI3K, FAK and SRC tyrosine kinases before miR-204 restoration effectively inhibits VM. Luciferase reporter assay confirms that PI3K, FAK and SRC genes are targets of miR-204. In vivo studies using tumor xenografts in mice model showed that miR-204 inhibited the formation of VM which was associated to decreased tumor growth and inhibition of metastasis. These data highlighted the importance of RNA-based multi-targeted therapies as an attractive approach in cancer. Also our findings provide novel potential targets for metastasis and VM intervention with therapeutic applications in breast cancer.
Title: Tβ4 expression in cancer-associated endothelial cells enhances progression of invasive breast cancer

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Body: Background: Invasive breast cancer is a highly aggressive primary breast tumour with poor prognosis. As tumour angiogenesis is exhibited by invasive breast cancer, anti-angiogenic therapy has been intensively evaluated over the past decade. However, clinical studies were disappointing. Thymosin β4 (Tβ4), a multi-functional peptide, is associated with induction of angiogenesis, but the role of Tβ4 in tumorigenesis of invasive breast cancer is unknown. We tested whether Tβ4 inhibition could be a new target involved in tumour development and tumour angiogenesis for treatment of invasive breast cancer.

Methods: We have adapted a 3D co-culture system to acquire invasive breast cancer cells-associated endothelial cells (ECs), which is followed by characterising the differential gene expressions by PCR-based subtraction analysis. We performed Western blot analysis on the protein samples obtained from ECs-stimulated by invasive breast cancer cells. Expression of Tβ4 in invasive breast cancer was assessed by qPCR in the breast tissues (tumour, n=119; background, n=55), and protein expression confirmed by immunohistochemical examination in an invasive breast cancer tissue microarray. Finally, mice breast cancer xenografts plus non-invasively photoacoustic microscopy were used to analyse the effect of Tβ4 knockdown on the tumorigenesis of invasive breast cancer.

Results: We found that invasive breast cancer cells could stimulate an increase in Tβ4 expression in microvascular ECs. High Tβ4 levels were strongly associated with high malignant invasive breast cancer and poor clinical outcome. In vivo study showed that siRNA targeting Tβ4 blocked growth of invasive breast cancer in mice. Photoacoustic imaging revealed that knockdown of Tβ4 elicited anti-breast cancer growth, in part, through disruption of tumour vasculature. Our results demonstrate that endothelial cell-derived Tβ4 enhances the progression of breast cancer by up-regulating tumour angiogenesis and it indicates that a high level of tumour stromal Tβ4 is an independent predictor of poor outcome.

Conclusions: Inhibiting endothelial Tβ4 could be a new therapeutic target in anti-angiogenic strategy for treatment of invasive breast cancer.
Intrinsic heterogeneity of triple-negative breast cancer cells triggers vascular mimicry in 3D matrigel matrix environment

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Body: Within the same tumor microenvironment phenotypic and functional heterogeneity arise among cancer cells as a consequence of genetic change, environmental differences, and reversible epigenetic changes in cellular properties. However, it is thought that cancer stem cells are drivers of drug resistance and metastasis. Individual tumor cells growing in culture also display heterogeneity in their intrinsic ability to progress and metastasize. It remains unclear whether intrinsic and extrinsic heterogeneity contribute to the emergence of distinct progressive phenotypes that contribute more to cancer stem cells to disseminate. To this study, we have examined the ability of matrigel to stimulate complex cell behavior that is a consequence of its heterogeneous composition. We have observed that mixing matrigel with metastatic triple negative breast cancer MDA-MB-231 cells, which mimic in vivo tumor microenvironment, around 80-90% cells created network like structures resembling a clinical phenotype known as vascular mimicry (VM) and around 10-20% cells form spheroids. BT549 another triple-negative breast cancer cells also responded similarly, forming cellular networks and spheroids when mixing with matrigel. Since CD44, a marker of epithelial-to-mesenchymal transition has shown enhances tumor aggressiveness by promoting cell plasticity, we decided to examine CD44 expression in MDA-MB-231 cells grown in 3D matrigel matrix environment. We have observed that VM forming cells are showing CD44 positive staining compared to spheroid forming cells which showed negative staining in formaldehyde-fixed 3D matrigel culture of MDA-MB-231 cells, while both group of cells stained positive for VEGFC. Next, we sought to isolate two phenotypically different groups of cells (VM and Tumorsphere forming cells) from the 3D matrigel culture by using microscopic suction procedure for gene expression analysis by qPCR. Our gene expression data suggested that VM forming cells have more expression of VM inducer genes such as CD44 and HIF1α compared to spheroid forming cells isolated from the same 3D matrigel culture of MDA-MB-231 cells. Spheroid forming cells express significant level of endothelial cell adhesion marker, CD31 compared to VM forming cells. Epigenetic mechanisms mediated suppression of tumor suppressors or anti-angiogenesis marker genes are hallmark of VM formation and cancer progression, we examine whether re-expression of those genes with Entinostat (MS-275), a selective inhibitor of class I histone deacetylase (HDAC) can abolish VM structures in 3D matrigel cell culture. Data suggested that MS-275 treatment in 3D culture drastically reduced VM structure by epigenetically re-expression of anti-angiogenic genes; SERPINF1, THBS1 and THBS2 and tumor suppressor genes; APC, PTEN and p21. While MS-275 treatment also downregulated Vimentin, VEGF-A and CD44. Our results suggest that the VM phenotype arises in a subpopulation of cells from a conserved transcriptional response in 3D matrigel environment. Epigenetically re-expression of anti-angiogenic gene expression could be a mechanism to control VM formation in triple-negative breast cancer cells.
2017 San Antonio Breast Cancer Symposium

Publication Number: P5-09-04

Title: The combination of PI3K/mTOR inhibitor and endostatin exerts synergistic anticancer activity against triple-negative breast cancer in vitro and in vivo

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Body: Background and Aims. Triple-negative breast cancer (TNBC) accounts for 15% of invasive breast cancer, which has no effective molecular targets and unfavourable prognosis. We investigated the effects of NVP-BEZ235, a novel dual PI3K/mTOR inhibitor, alone and in combination with an angiogenesis inhibitor Endostatin in the orthotopic TNBC model and explored its anti-tumor and anti-angiogenesis mechanisms.

Methods. MTT assays were performed to assess sensitivity of the cells to the drug. Tumor growth and survival studies were performed in orthotopic xenografts. Additionally, Immunohistochemistry for VEGF and MVD (CD34) was performed using the EnVision/HRP technique. Serum VEGF was detected using ELISA method.

Results. BEZ235 effectively inhibited cell proliferation in vitro and provided additive effects in combination with Endostatin. Treatment with BEZ235 and Endostatin resulted in inhibition of tumor growth and prolongation of survival time of tumor-bearing mice in vivo. Immunohistochemical analysis revealed that intratumoral proliferation and angiogenesis was significantly suppressed. Furthermore, the finding of angiogenesis inhibition was also supported by measuring the number of Serum VEGF.

Conclusion. Our findings suggest that BEZ235 exerts antitumor effects against TNBC and enhances effects of Endostatin through inhibition of cell proliferation and tumor angiogenesis. This approach may represent a promising combination targeted therapy for TNBC treatment.

Key words: PI3K/mTOR inhibitors; Endostatin; Angiogenesis; Triple-negative breast cancer.
Cellular senescence within HER2-amplified breast cancer: Potential implications for breast cancer immune surveillance and HER2-targeted therapy resistance

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Body: Background: Oncogene-induced senescence is considered as a barrier to tumor progression that arrests cells at risk for malignant transformation. Nevertheless, numerous findings demonstrate that senescent cells may also have the opposite function and promote tumor progression through the release of multiple factors called the senescence-associated secretory phenotype or senescence secretome. It is likely that the composition and the physiological consequences mediated by the senescence secretome are dependent on the oncogenes that trigger the senescence program. Breast cancer represents a heterogeneous disease that can be divided into breast cancer subtypes caused by different subsets of genetic and epigenetic abnormalities. Therefore, tumor initiation and progression of breast cancer subtypes is triggered by variable oncogenic stimuli, and differences in the senescence secretomes within breast tumors might be responsible for tumor initiation, progression, metastasis and therapeutic response. Beside many studies concerning the role of senescence as a barrier to tumor progression using murine xenograft models very few investigations have been performed to elucidate how often senescent tumor cells appear within untreated human tumors, and if present whether these senescent tumor cells may play a role in disease progression, cancer immunosurveillance and therapy resistance.

Method: In the present study we analysed the appearance of senescent cells within invasive human breast cancers from 129 untreated patients. Cellular senescence was detected by the use of SAβ-gal staining and by immunohistochemical detection of p16, p21, p53, Ki67 and lamin B1.

Results: Detection of cellular senescence by the use of SAβ-gal staining and detection of p16, p53, Ki67 and lamin B1 within invasive breast carcinomas indicate that senescent tumor cells varies strongly according to the breast cancer subtype. The highest percentages of senescent tumor cells exist within in the HER2-positive and luminal A breast carcinomas whereas no or very few senescent tumor cells were found in triple negative breast tumors. Based on these findings we suggest that the composition of secretomes released by senescent tumor cells from different breast cancer subtypes might be very distinct in respect to their ability to recruit immune cells, which can eliminate senescent tumor cells on one hand and regulate tumor growth, immune surveillance and therapy resistance on the other.

Conclusion: Further characterization of senescent secretomes from HER2 and other breast cancer subtypes and their potential role in tumor progression, immune surveillance and therapy response might be warranted for the understanding of cancer biology as well as prognostic and therapeutic applications.
Title: High expression of BAD, PUMA, BOK and TRADD mRNA is associated with higher overall survival in ER+ and PR+ breast cancer patients

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Body: Background: Apoptosis signalling is controlled by a complex interaction of pro- and anti-apoptotic BCL2 proteins and its dysregulation is believed to be a major contributor to therapy responses and resistance in cancer. We previously demonstrated that inhibition of cell death in cancer cell is associated to poor outcome in colorectal cancer (Lindner et al., Cancer Res, 2012) and to lower cell death in triple negative breast cancer (TNBC) cells in vitro (Lucantoni et al., in review). In ER+ breast cancer, the anti-apoptotic protein BCL2 is commonly overexpressed, but its expression is associated with improved clinical outcome. The aim of this study was to assess whether system modelling of BCL2 protein interactions stratifies low- and high-risk breast cancer patients, and to determine the contribution of apoptosis signalling in different molecular subtypes of breast cancer.

Methods: Protein levels of BAK, BAX, BCL2 and BCL(X) were determined in fresh frozen, TNBC samples from the BREAST-PREDICT Irish Cancer Society Collaborative Research Centre cohort, using HeLa cells as standard in which absolute protein levels were previously determined. Clinical, protein level and gene expression datasets of 845 invasive breast carcinoma patients were accessed from The Cancer Genome Atlas project, and BCL2 protein profiles were calculated by linear regression based on the BREAST-PREDICT cohort. In both cohorts, profiles were used to calculate the stress dose required to induce mitochondrial apoptosis ($\eta$).

Results: In contrast to experiments with TNBC cells in which a high $\eta$ indicated lower rates of cell death in vitro (Lucantoni et al., in review), we found that in breast cancer patients, $\eta \leq 0$ was associated with lower overall survival (OS) compared to $\eta > 0$ (HR 2.1, 95%CI 1.3-3.3, p < 0.01). $\eta > 0$ was associated with lower levels of cleaved caspase 7 compared to $\eta \leq 0$ (ANOVA & Tukey post-hoc; p < 0.1). Cleaved caspase 7 levels > mean were associated with improved OS compared to levels $\leq$ mean (HR 0.4, 95%CI 0.3-0.7, p = 0.001). High values of $\eta$ were significantly associated with lower proliferation in ER/PR+ cancer (ANOVA & Tukey post-hoc; p < 0.01), but not in HER2+ or TNBC. Next we performed hierarchical cluster analysis (ConsensusClusterPlus; Monti et al, Machine Learning, 2003) with 61 additional mRNAs and proteins which are not implemented in our systems modelling approach. We found a subgroup with high BAD, PUMA, BOK and TRADD mRNA expression levels in ER/PR+ breast cancer patients, independently of the value of $\eta$. ER/PR+, but not HER2+ or TNBC, patients with an averaged expression of these 4 mRNA $> \text{mean}$ had significant higher OS compared with patients with an averaged expression $\leq \text{mean}$ (HR 0.4, 95%CI 0.2-0.9, p = 0.02).

Conclusions: Impairment of apoptosis assessed solely on the levels of BAK, BAX, BCL2 and BCLX(L) proteins were not sufficient as prognostic marker in breast cancer patients. However, our analysis suggest that patients with ER/PR+ cancer cells potentially "primed" towards apoptosis - via the expression of pro-apoptotic BAD, PUMA, BOK and TRADD - had a more favourable clinical outcome compared to patients with cancer cells lacking this priming.
Title: The accuracy of nomograms based on large dataset using clinico-pathologic variables for prediction of oncoype DX breast cancer recurrence score

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Body: Purpose
The 21 gene expression assay, Oncotype DX (ODX) is costly (the current estimated cost is ~$4000), and were tested in only approximately one-third of breast cancer patients who meet the inclusion criteria in the United States. Recent study of University of Tennessee Medical Center reported a novel nomogram for prediction of ODX recurrence score using clinico-pathologic data, which was based on a large dataset from the National Cancer Data Base (NCDB). The purpose of this study was to confirm that the nomogram could predict ODX score group accurately with Korean data, which is different from the NCDB in terms of ethnicity.

Methods
A retrospective review was performed with 218 patients who received breast-conserving surgery or mastectomy at Samsung Medical Center and were tested with the ODX from April 2011 to January 2017. An online nomogram calculator, provided by the University of Tennessee Medical Center on their website was used in order to calculate the probability of a low-risk or a high-risk ODX score for each patient. The cut-off value for predictive accuracy of the nomogram was set same as the original study.

Results
With the cut-off value as 90% for inspection of the commercial ODX score, high-risk group showed 33.3% positive predictive value (PPV) and low-risk group showed 68.7%. With the cut-off value as 86% for TAILORx score, PPV was 66.7% for high-risk group, and 38.9% for low-risk group. The sensitivity of the nomogram for the low-risk group commercial ODX score was 96.8%, and 33.3% for the TAILORx score. For the high-risk group, sensitivity was 20% for the commercial ODX score, and 16.7% for the TAILORx score.

Conclusions
Although the nomogram for prediction of ODX score was based on a large dataset of NCDB, it could not be generalized to patients in Asia. Further studies based on large data from different ethnicities should be performed to develop the nomogram for patients worldwide.
Predicting local recurrence in patients treated for ductal carcinoma in situ of the breast (DCIS)

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Body: Background: Ductal carcinoma in situ (DCIS) of the breast represents a heterogeneous group of precursor, non-invasive breast lesions. Currently we lack accurate tools to stratify DCIS patients according to inherent risk of in-breast tumour recurrence (IBTR) or progression to invasive breast cancer (IBC). Most DCIS patients are treated by breast-conserving surgery (BCS), followed by whole-breast radiotherapy (RT) for the majority of high-grade DCIS. The aim of this study was to identify novel biomarkers which predict recurrence after BCS +/- RT.

Methods: A single institution study of 466 consecutive patients (median age 61, range 35-94) with DCIS treated by BCS between 2000 and 2010 was carried out. 271 patients with grade 3 DCIS received RT and 155 with grade 1/2 DCIS did not receive RT. For biomarker discovery, a case-control matched series of 200 patients (mean age = 61, range = 36-84) from the above audit that met the following criteria was selected:

· 120 with low/intermediate-grade DCIS treated with BCS alone: 30 have recurred, 90 patients matched 3:1 have not recurred by 10 years.
· 80 with high-grade DCIS treated by BCS plus RT: 20 have recurred, 60 patients matched 3:1 have not recurred by 10 years. Median follow-up was 7.4 years. RNA has been extracted and Affymetrix Clariom S whole-genome analysis has been performed and is currently being analysed.

Results:

In the cohort of 466 patients, 271 patients with high grade DCIS had BCS plus RT. Actuarial IBTR and IBC-IBTR in this group were 10% and 4% at 5 years and 18% and 6% at 10 years, respectively. 155 patients with low/intermediate grade DCIS had BCS alone. Actuarial overall IBTR and IBC-IBTR in this group were 6% and 2% at 5 years and 13% and 2% at 10 years respectively. In the high-grade, RT treated group, lesion size (P<0.001, P=0.003), presence of comedo necrosis (P=0.018, P=0.025) and the Van Nuys Prognostic Index (VNPI) (P=0.02, P=0.004) were significantly associated with overall IBTR and DCIS-IBTR. No factor was significantly associated with IBS-IBTR in the high grade group and no factor predicted for any IBTR in the low/intermediate group.

Full genomic analysis of the 240 patient case-control matched cohort is underway and will be presented.

Discussion:

· This is the first DCIS biomarker discovery study using whole genome analysis and the matched cohort design looking separately at BCS + RT for high-grade DCIS and BCS only for low/intermediate grade DCIS.
· Clinical parameters alone may have insufficient sensitivity to identify high-grade, RT-treated patients at risk of developing IBC-IBTR.
· While recurrence rates in the low/intermediate grade DCIS group are lower than in the high-grade group, some patients do recur and there is a need to develop new tools which can identify low grade patients with a sufficiently high risk of recurrence to warrant additional treatment.
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Title: Tumor CXCL16/CXCR6 expression and soluble CXCL16 in HER2+ breast cancer (BC)

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Body: Background: CXCL16 is a pro-inflammatory chemokine associated with chemotaxis of CXCR6-expressing lymphocytes (T cells, NKT cells, NK cells) and the promotion of breast cancer cell proliferation, migration, and invasion in vitro. CXCL16 exists in a transmembrane or a cleaved, soluble form. There is limited clinically relevant data available on the CXCL16/CXCR6 signaling axis in HER2+ BC. This preliminary study examines tumor CXCL16 and CXCR6 mRNA expression and patient outcome in publicly available datasets and examines soluble CXCL16 in the plasma of 32 HER2+ BC patients enrolled in ICORG 10-05 (neo-adjuvant chemotherapy (docetaxel/carboplatin) +/- trastuzumab, lapatinib or trastuzumab/lapatinib).

Methods: CXCL16 and CXCR6 mRNA expression was interrogated in publicly available datasets using BreastMark, a web-based tool for preliminary assessment of putative biomarkers in breast cancer. A median cut-off was used for all analyses. Pre-treatment and post-treatment (2 weeks pre-surgery) blood samples were collected from patients enrolled in ICORG 10-05. Plasma CXCL16 levels were determined by Luminex xMAP assay. Pre- and post-treatment levels of CXCL16 were compared directly or based on response (pathological complete response, CR, n=14 or non-pathological complete response, nCR, n=18). Stromal lymphocyte (SL) and tumor-infiltrating lymphocyte (TIL) levels were determined from Haematoxylin and Eosin-, AE1/AE3- and CD45FFPE- stained formalin-fixed paraffin embedded tissue. Pre-treatment lymphocyte levels were correlated with pre- and post-treatment levels of CXCL16 (Pearson's product-moment correlation).

Results: In BC as a whole, analysis of publicly available datasets reveals tumor CXCL16 expression is not associated with outcome (n=1238, HR=0.9953, p=0.9516) but high tumor CXCR6 expression is (n=2652, HR=1.127, p=0.0476). Within a HER2+ cohort, inverse correlative analysis suggests high CXCR6/low CXCL16 tumor expression is significantly associated with a worse outcome (n=61, HR=2.731, p=0.01). For ICORG 10-05 patients, circulating CXCL16 plasma levels were significantly higher post-treatment (p<0.0001). The magnitude of this increase was significantly greater in CR than nCR patients (p<0.009). Post-treatment circulating CXCL16 levels negatively correlate with pre-treatment total (SL+TIL) lymphocyte counts (correlation coefficient -0.50 (CI -0.75 - -0.13), p=0.01) in ICORG 10-05 patients. SL and total lymphocyte (SL+TIL) counts were higher in CR (n=13) vs. nCR (n=13) patients but the difference was not significant (SL, p=0.22; SL+TIL, p=0.29).

Conclusions: Our preliminary results suggest tumor levels of CXCL16/CXCR6 are associated with patient outcome and circulating levels of soluble CXCL16 are altered by treatment and correlate with tumor immune infiltrate in HER2+ BC patients. Further examination of tumor CXCL16/CXCR6 expression and circulating CXCL16 as potential biomarkers of response is warranted in a larger cohort of HER2+ BC patients.
Title: Therapy-induced priming of natural killer cells predicts patient-specific tumor rejection in multiple breast cancer indications

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Body: Background: Predicting patient-specific clinical response to anticancer therapy is the holy grail of treatment-selection. It is now clear that response or resistance to therapy depends on the heterogeneous tumor microenvironment, which is comprised of malignant cells, normal stroma, soluble ligands, and tumor-immune contexture; attributes that are unique to each individual patient. This is particularly true for emerging anticancer drugs, such as immune checkpoint inhibitors, which recalibrate the body’s own immune defense largely by modulating exhaustion of cytotoxic lymphocytes including T cells and natural killer (NK) cells. However, clinical response to therapy varies enormously. There is a critical gap in our understanding for the mechanisms that drive response or resistance to conventional drugs and immunotherapies at the individual patient level.

Methods: Here, we used a fully patient-autologous, clinically-validated ex-vivo tumor model that recreates and preserves the native, patient tumor microenvironment (CANscriptTM), which incorporates an algorithm-driven method to predict clinical response to therapy (M-Score). Utilizing tissue from patients diagnosed with luminal, HER2 positive, and triple-negative (ER- PR- HER2-) breast cancers (N=10), we studied phenotypic alterations to the tumor-immune contexture under pressure of conventional standard-of-care regimens and immunotherapies including immune-checkpoint inhibitors, ex-vivo. To do this, we used a comprehensive panel of immunological assays to evaluate changes in cytotoxic lymphocytes by flow cytometry and multiplex immunohistochemistry (i.e. CD56, MHC class 1A/B, NKG2D/C, CD8, CD3, PD-1, CTLA-4, TIM-3, LAG-3, 4-1BB, granzyme A/B). In addition, we used multiplex cytokine analysis to study the soluble components of the tumor microenvironment.

Results: We identified that tumor response, predicted by M-Score, correlates to increased infiltration of NK cells, which associated a pro-inflammatory cytokine signature from the tumor microenvironment. Interestingly, these evidences were concordant with induction of the tumor-expressing biomarker MICA/B, which is known to attract and recruit active NK cells. Furthermore, we determined that therapy-induced expression of protein biomarkers associated with NK cell exhaustion inversely correlated to the expression of cytotoxic granzyme B in the tumor microenvironment.

Conclusions: Taken together, these data demonstrate an integral role that NK cells contribute to the antitumor effect of therapy including conventional and immuno-modulatory drugs. It further demonstrates how a novel ex-vivo platform can be harnessed to study the mechanisms of response and resistance, which couldn’t otherwise be known in a drug naïve state. Such an advance in our preclinical methods to study anticancer drugs at the individual patient level can help guide treatment decisions for clinicians while simultaneously functioning as a platform to study clinical efficacy of novel and emerging agents.
Title: The joint effect of modifiable risk factors on risk of invasive breast cancer among Canadian women

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Body: Background
Although several studies have suggested that obesity and other modifiable factors influence breast carcinogenesis, there is limited evidence on the combined effect of these exposures on risk of breast cancer. Thus, to gain further insight into whether modifiable risk factors may operate collectively to alter risk of breast cancer, we assessed the association of a healthy lifestyle index (HLI) score (a combination of diet, physical activity, smoking, alcohol consumption and anthropometry) with risk of invasive breast cancer among women in the Canadian Study of Diet, Lifestyle, and Health (CSDLH).

Method
We performed a case-cohort study using a cohort of 39,532 female participants in the CSDLH who were recruited between 1995 and 1998. The study included an age-stratified subcohort of 3,027 women and 1,018 incident invasive breast cancer cases. Lifestyle and dietary information was collected at baseline using self-administered questionnaires. We estimated hazard ratios (HR) and 95 % confidence intervals (CI) for the association of the HLI score and the individual HLI components with risk of breast cancer using Cox proportional hazards regression modified for the case cohort design.

Results:
Every unit increase in the HLI score was associated with a 3% reduced risk of breast cancer (HR: 0.97; 95% CI: 0.95-1.00). When considering quintiles of the HLI score, those in the highest quintile had a 26% lower risk for breast cancer compared to those with HLI score in the lowest quintile (HR_q5 vs q1: 0.74; 95% CI: 0.59-0.93). In analyses stratified by menopausal status, there was a similar inverse association between the HLI score and risk of breast cancer among postmenopausal women (HR: 0.97; 95% CI: 0.94-1.00) and a tendency towards an inverse association among premenopausal women (HR per unit increase in the HLI score: 0.98; 95% CI: 0.95-1.01). Among the individual components of the HLI score, there was some suggestion of a reduction in risk of breast cancer in association with a relatively high physical activity level in the overall study population (HR_q5 vs. q1: 0.81: 95% CI: 0.63-1.04, p trend: 0.08), but consuming a healthy diet, smoking, having high alcohol consumption and being obese were not associated with altered risk of breast cancer.

Conclusion: Our findings indicate that adherence to a healthy lifestyle may be associated with reduced risk of breast cancer. These findings suggest the need to further investigate how modifiable risk factors may act jointly to contribute to the development of breast cancer. Such knowledge may have important implications for the development of interventions designed to promote a healthy lifestyle to aid in the primary prevention of breast cancer.
Title: B-vitamin intake from diet and supplements and breast cancer risk in middle-aged women: Results from the prospective NutriNet-Santé cohort

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Body: Experimental studies suggest a protective effect of B-vitamins on breast cancer risk, potentially modulated by alcohol intake. However, epidemiological studies are limited, especially regarding non-folate B-vitamins. Furthermore, few of them included quantitative assessment of supplemental intake. This prospective study aimed at investigating the associations between intakes of B-vitamins (dietary, supplemental, total) and breast cancer risk. 27,853 women aged ≥45y from the NutriNet-Santé cohort (2009-2016) were included, with a median follow-up time of 4.2 years. Dietary data were collected using repeated 24h records. A specific questionnaire assessed dietary supplement use over a 12-month period. A composition database of 8000 supplements was developed. Associations were characterized by multivariable Cox models. 462 incident breast cancers were diagnosed. Dietary (HRQ4 vs Q1 = 0.74 (0.55, 0.99), P-trend = 0.05), supplemental (HRQ4 vs Q1 = 0.61 (0.38, 0.98), P-trend = 0.05) and total (HRQ4 vs Q1 = 0.67 (0.50, 0.91), P-trend = 0.01) pyridoxine intakes were inversely associated with breast cancer risk. Total thiamin intake was borderline inversely associated with breast cancer risk (HRper 1-unit increment= 0.78 (0.61, 1.00), P = 0.05). Statistically significant interactions between alcohol consumption and B-vitamin (thiamin, riboflavin, niacin, pantothenic acid, pyridoxine, folate, and cobalamin) supplemental intake were observed, the latter being inversely associated with breast cancer risk in non-to-low alcohol drinkers but not in higher drinkers. This large prospective study, including quantitative assessment of supplemental intake, suggests a potential protective effect of pyridoxine and thiamin on breast cancer risk in middle-aged women.
The impact of bariatric surgery on mammographic breast density

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Body: Background:
Bariatric surgery decreases breast cancer risk, but its impact on mammographic findings is not well understood. Obesity and breast density both increase breast cancer risk, but paradoxically are inversely related. We investigated how mammographic density changes after bariatric surgery, and whether or not that change is related to amount of weight loss.

Methods:
We reviewed records for 349 prospectively collected patients who underwent bariatric surgery between 2013-2015, and identified 45 women with pre- and post-operative screening mammograms within 1.5 years of surgery. We recorded body mass index (BMI), Breast Imaging-Reporting and Data System density, and calculated excess BMI loss. Data were analyzed in Stata 14.2.

Results:
Average age was 54 years, mean pre-operative BMI was 44 (range 36-72), and mean percentage excess BMI lost was 73% at 1.3 years. One third had a change in mammographic breast density, which increased 93% of the time (p<0.001). Amount of weight loss was not associated with density change; in fact, weight loss was lower in those with a density change than in those without a density change (68% versus 75% excess BMI lost).

Conclusions:
The majority of women with a mammographic change had an increase in breast density, despite bariatric surgery being associated with reduced breast cancer risk. Interestingly, the amount of weight loss was not associated with change in breast density. These findings suggest the metabolic effects of bariatric surgery have an effect on breast cancer risk independent of BMI reduction. Future work will include studying mammographic changes associated with non-surgical weight loss.
Title: Effects of a 16-week combined aerobic and resistance exercise intervention on metabolic syndrome in overweight/Obese Hispanic breast cancer survivors

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Body: Purpose. Metabolic syndrome (MetS) is associated with increased risk of cardiovascular disease, type 2 diabetes, and possibly cancer recurrence, and is higher in breast cancer survivors than age-matched postmenopausal women. Further, MetS is 1.5 times more prevalent in Hispanic women (>40 years of age) than in non-Hispanic Whites and African Americans, thereby increasing the need to attenuate MetS in Hispanic breast cancer survivors (HBCS). This study examined the effects of a 16-week combined aerobic and resistance exercise intervention on MetS in overweight and obese HBCS.

Methods. This pre-planned sub-analysis included 60 sedentary HBCS (BMI³25 kg/m²) from our larger MetS trial. HBCS were randomized to the exercise intervention (EXE; n=30) or usual care (UC; n=30). The EXE group participated in 3 supervised exercise sessions per week for 16 weeks. Aerobic exercise was performed at 65-85% heart rate maximum for ~30 minutes. Resistance exercise was performed in circuit-fashion with 3 sets of 10-15 repetitions including upper and lower body exercises at 65-85% 1-repetition maximum. The UC group was asked not to increase their current exercise levels during the study period. Participants were tested for MetS (blood pressure, waist circumference, fasting blood glucose, HDL-C, and triglycerides) at baseline, within one week following the 16-week study period, and at 12-week follow-up for the EXE group only. Fasting blood samples were used to measure glucose, HDL-C, and triglycerides. Waist circumference was measured at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest using a fabric tape measure. Blood pressure was measured with an automated sphygmonanometer. Body composition was assessed via dual energy X-ray absorptiometry.

Results. At baseline, 82% (overall and by group) of the HBCS met the criteria for MetS. There were no significant group differences in the MetS variables between the EXE and UC groups at baseline (p>0.01). Post-intervention, all MetS components were significantly lower in the EXE group than the UC group (p<0.01) and only 15% of participants in the EXE group met the criteria for MetS, representing a 67% absolute decrease. This is in comparison to 84% of participants in the UC group. Body fat mass decreased by 10% during the 16-week EXE period, compared to a 2% increase in the UC group (p<0.01). MetS changes remained significantly improved in the EXE group when fat mass was included as a covariate in the statistical model. At the follow-up assessment in the EXE group, all MetS variables remained significantly improved compared to baseline (p<0.01) and were not significantly different post-intervention (p>0.25) despite slight increases (<2%) in waist circumference and triglyceride levels.

Conclusion. This is one of few exercise trials in minority BCS and the first study to target MetS with exercise in HBCS. This 16-week supervised combined aerobic and resistance exercise intervention reduced MetS in sedentary, overweight and obese HBCS. Reductions in MetS components were maintained after completion of the intervention, suggesting the benefits of the intervention on MetS were sustainable in the absence of a supervised intervention.
Title: Chemoprevention utilization in patients with a history of atypical hyperplasia, atypical lobular hyperplasia, or lobular carcinoma in-situ: A retrospective chart review of patients diagnosed at an urban hospital with a large minority patient population

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Body: Background: The Breast Cancer Prevention Trial (BCPT) and the Study of Tamoxifen and Raloxifene (STAR) trial showed that chemoprevention can reduce the risk of invasive breast cancer by nearly 50%. Despite these results, studies have shown that while an estimated 2 million women in the United States are eligible for chemoprevention, actual acceptance of these medications is low. Improving chemoprevention utilization rates hinges on better understanding current rates of utilization and factors affecting patient acceptance. Reported rates and barriers to chemoprevention use may not accurately reflect true utilization patterns in lower socioeconomic, minority patient populations. The aim of this IRB approved retrospective study was to characterize the rate and factors associated with chemoprevention use in patients with a diagnosis of atypical ductal hyperplasia (ADH), atypical lobular hyperplasia (ALH), or lobular carcinoma in-situ (LCIS) at an urban hospital with a high minority population.

Methods – A retrospective chart review was performed for all diagnoses of ADH, ALH, and LCIS made at the University of Maryland Medical Center between the years 2005-2015. Concurrent DCIS or invasive cancer were excluded. Demographic and clinical information including age, race, education, GAIL score, BMI, and use of chemoprevention was recorded. Univariable and multivariable logistic regression were performed to identify factors associated with chemoprevention discussion and use.

Results – 301 diagnoses of ADH/ALH/or LCIS were obtained and 127 women were eligible for analysis. The median age was 53 years old with 47% of patients being premenopausal. The majority were African-American (65%) and 51% had a high school degree or less. The median 5 year risk for developing breast cancer based on the GAIL model was 2.4%. The chemoprevention utilization rate for our patient population was 28% (n=34). Race, menopausal status, and breast density were not associated with chemoprevention discussion or use. We found that patients were more likely to have a chemoprevention discussion with their provider if they were older (p=0.03) or if they were referred to medical oncology (p<0.001, Fisher's exact test).

Conclusions - Our study evaluated chemoprevention use in an understudied predominantly African-American patient population. We found a higher rate of chemoprevention utilization (28%) compared to previously reported rates. Age and medical oncology referral had a significant impact on provider-patient chemoprevention discussion. Though limited due to small sample size, our study nonetheless provided thought provoking results. Older patients may be at higher risk for developing breast cancer, however, it is important to consider that younger patients with risk factors may have a more favorable endocrine therapy benefit-risk ratio. In addition, our results highlight the importance of encouraging all physicians who are involved in women's' breast health to have a chemoprevention discussion with eligible patients, or for these physicians to refer patients to a medical oncologist for further discussion.
Title: The use of a behavior-modification clinical solution application to improve breast cancer survivors' accountability and health outcomes

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Body: Background: Studies have demonstrated that obesity increases the risk of breast cancer recurrence and death in survivors but only 34% of breast cancer survivors engage in the recommended level of physical activity. This low percentage is related to a lack of accountability and motivation. We hypothesize that using a mobile application (app) incorporating the concept of cognitive-behavioral therapy and dietary and physical activity recommendations will improve breast cancer survivors' accountability and help them reach their personalized health goals; specifically with diet and exercise. Methods: We have created an app, METHODIST HOSPITAL CANCER HEALTH APPLICATION (MOCHA) for the purpose of patient self-reinforcement through the daily accounting of activity and nutrition as well as group feedback and direct interaction with clinical dietician. To test the MOCHA app's feasibility, we enrolled 33 breast cancer survivors with a body mass index (BMI) over 25 who were at least 6 months post active treatment (surgery, chemotherapy, or radiation) for a 4 week feasibility trial. During these 4 weeks, the users used the app to track wellness (mood, sleep or pain), diet (calorie intake) and exercise (walking or steps). Our primary objective was to demonstrate adherence, defined as the number of days recorded on MOCHA during week 2 and 3 of the 4 week study period (14 days). A registered dietitian assigned personalized goals for each user and monitored their usage of the app and followed the progress of their goals. Additionally, the dietitian sent daily push notifications to encourage the user to stay on track. Results: Our results suggests a correlation between utilization of the app and achievement of the goals of weight loss and increased motivation to exercise. The average number of daily uses of the app was approximately 3.76 (0-12) and 50% of enrolled users lost average of 2.14 lbs (0-6lbs) weight during this short 4 week study; preliminary correlation analysis suggest a correlation coefficient of -0.42 between these two variables. This is noteworthy as traditionally we would expect weight increase in this group of users. Our secondary objective was to determine MOCHA's usability using System Usability Scale (SUS) scale. Our average score on the SUS scale is 77%, which is above average. Lastly, users have stated that access to the dietitian in the app improves their food choices and accountability. Conclusion: This study provides essential data that emphasizes the importance of using technology to improve patients' goal adherence by providing real-time feedback and accountability with their healthcare team. Most health mobile apps focus on data acquisition but without the engagement of the health care team, this aspect differentiates MOCHA from the other apps. Our future directions will focus on using our MOCHA app in breast cancer survivors in a long term behavior modification study.
Title: Telapristone acetate abrogates PR-dependent paracrine-mediated mammary cell proliferation

Oukseub Lee¹, Limin Sun¹, Lindsey C Karavites², Susan E Clare¹ and Seema A Khan¹.¹Northwestern University, Chicago, IL and ²Mount Sinai Hospital, Chicago, IL.

Body: Background: Blockade of the progesterone (P)-progesterone receptor (PR) axis is a novel but untested strategy for breast cancer prevention. We report preclinical data evaluating telapristone acetate (TPA) compared with mifepristone (MFP), the prototype PR-antagonist. We hypothesize that the progesterone-PR blockade by TPA will inhibit PR-dependent paracrine expression (RANKL, WNT4, ID4, and calcitonin) attenuating cell proliferation and abrogating side branching and alveoli formation of mammary glands induced by hormones similar in extent or superior to MFP.

Methods: Adult virgin FVB mice at 12 weeks of age were randomized to four treatment groups: no treatment control, EP (0.3 mg E + 30 mg P), EP + TPA (30mg), and EP + MFP (30mg). Hormone and drug pellets were subcutaneously implanted in flank area between the neck and shoulder. After 28-day treatments, the mice were euthanized to collect mammary glands, and processed as mammary whole mounts and as formalin-fixed paraffin embedding (FFPE) specimens. We evaluated cell proliferation (Ki67) by immunohistochemistry. Total RNA was extracted from FFPE specimens and the Nanostring nCounter assay was utilized to assess paracrine gene expression. The Mann Whitney test was used to calculate statistical significance (p<0.05).

Results: We observed a considerable increase in side branches and alveoli in EP treatment group compared to the controls. The growth of the mammary gland stimulated by EP treatment was corroborated by a significant increase in Ki67 compared to control mice (median 41% vs 24%, respectively, p < 0.0001). Both TPA and MFP treatment abrogated side branching and alveoli formation and significantly reduced median Ki67 to 1/3 of that of control mice having endogenous estrus cycle hormone levels (24%, 8%, and 9% for control, EP+TPA and EP+MFP groups, respectively). TPA induced greater Ki67 reduction than MFP (7.5 ± 1.9% vs. 9.4 ± 1.9%, p= 0.04). As expected, EP treatment upregulated the RANKL expression compared to the control group while the administration of TPA and MFP significantly inhibited the RANKL expression stimulated by EP treatment. The mRNA expression measurement revealed that Rankl expression was 18 fold increased by EP treatment but completely inhibited by TPA and MFP treatment (p<0.001 for both drugs). Similarly the Rank expression was increased two-fold by EP treatment (p< 0.05) but down-regulated by TPA and MFP treatment (p<0.01 for both drugs). We observed significant upregulation of Wnt4, and Calca expression by EP treatment compared to controls (p<0.01 for both). Id4 expression showed the same trend but the change was non-significant. The addition of TPA completely opposed the hormone-stimulated increase of three paracrine molecules (Wnt4, Calca, and Id4) to a level below control group (p< 0.001 for all).

Conclusions: We have demonstrated that TPA abrogates cell proliferation induced by exogenous EP hormones in an ovary intact mouse model. The blockade of PR-P binding was evident by complete inhibition of paracrine expression (not only RANKL/RANK expression but also WNT4, Calcitonin, and ID4 expression). TPA was efficacious as MFP in opposing paracrine-induced mammary cell proliferation, and warrants further testing in a breast cancer prevention trial.
2017 San Antonio Breast Cancer Symposium

Publication Number: P5-14-02

Title: Effects of bazedoxifene and/or letrozole in the prevention of chemically-induced mammary cancers

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Body: Bazedoxifene is described as a third-generation selective estrogen receptor modulator (SERM) that has been approved in Europe for the prevention and treatment of postmenopausal osteoporosis. The agent has also been shown to have therapeutic activity against estrogen receptor positive (ER⁺) breast cancer. Our laboratory has evaluated bazedoxifene for chemopreventive efficacy against mammary cancers when given alone or in combination with the aromatase inhibitor letrozole. Initially, the agents were evaluated alone to determine effective chemopreventive doses in the methylnitrosourea (MNU) ER⁺ mammary cancer model using female Sprague-Dawley rats. Bazedoxifene was mixed directly into a standard (Teklad, 4% fat) diet, while letrozole was gavaged 7x/week (vehicle was ethanol: PEG 400, 10:90, v/v). When evaluated alone in rats receiving MNU at 50 days of age, bazedoxifene (started one week after MNU) at doses of 100 and 30 mg/kg diet decreased mammary cancer multiplicity by 93 and 88%, respectively. In a similar protocol, letrozole at a dose of 0.1 mg/kg BW/day reduced cancer multiplicity by 89%, while a dose of 0.05 mg/kg BW/day reduced the number by 47%. In a separate study evaluating lower doses of bazedoxifene (5 mg/kg diet) and letrozole (0.04 mg/kg BW/day), mammary cancer multiplicity was decreased by 73% by both agents. When the agents were given in combination at these lower dose levels, cancer number was reduced by 91%. It is obvious that bazedoxifene is as effective as letrozole in the prevention of ER⁺ mammary cancers. Furthermore, administration of the agents in combination suggests an additive effect. The large increase in body weight gain generally noted in letrozole treated rats was not observed in rats receiving bazedoxifene; of interest, rats receiving the combination also had no increase in body weights. Several published invitro studies suggested that bazedoxifene might also be effective in preventing ER⁻ mammary cancers; e.g., the agent is a novel inhibitor of IL-6/GP130 protein-protein interactions using multiple ligand simultaneous docking and drug repositioning approaches that result in apoptosis. In an initial study using MMTV/Neu mice (N=21/group) that received dimethylbenzantracene (DMBA), we showed that administering bazedoxifene (15 mg/kg diet) would reduce the mammary tumor multiplicity from 5.4/mouse in controls to 1.1/mouse (an 80% decrease). These studies suggested that bazedoxifene is superior to other similar agents in that both ER⁺ and ER⁻ mammary cancers are prevented at non-toxic doses. Supported by NCI contract HHSN261201200021I, Task Order HHSN26100007.
Title: A novel implant to deliver localized hormonal therapy to prevent and treat breast cancer

Pamela N Munster¹, Jeenah Park¹, Pujan Desai², Estelle Garcia³, Sarah Cheng³, Steven Greier², Nela Pawlowska¹, A Ray Chaudhuri¹ and Scott Thomas¹. ¹University of California, San Francisco, CA and ²University of California, Berkeley, CA.

Body: Most cancer treatment and prevention strategies include removal of the respective organ or systemic therapy. Early interception and cancer prevention is fraught with uncertainties in individual risk assessment and the absence of early surrogate markers to monitor efficacy. Hence, cancer prevention studies typically require large patient numbers. They are performed in unselected populations without clearly defined risk and benefits are often small or diluted. Hence, even successful strategies with documented benefit such as tamoxifen, have found only poor uptake in the at-risk population. Many women and providers are deterred by the low benefits to risk ratio of systemic tamoxifen exposure. The opportunity to selectively treat with an effective agent would limit the need for surgery and circumvent systemic exposure.

We propose a less toxic and less debilitating approach to prevent and treat early stage breast cancer by utilizing the slow release of anti-estrogens from silastic tubing as a local drug delivery device to the breast. Our in vitro and in vivo data demonstrate consistent release of active fulvestrant through at least 52 weeks. Extrapolating from the amount of residual drug left in the tubing after 52 weeks suggests that drug release could be maintained sufficiently to and beyond 5 years. Silastic tubing released fulvestrant at clinically relevant concentrations and associated with inhibition of ER signaling and cell proliferation in vitro. In vivo anti-tumor activity was comparable to systemic administration of the anti-estrogen. The silastic tubing preferentially delivered the anti-estrogen to mammary tissue with minimal accumulation in major organs and 20-fold lower concentrations in adjacent (abdominal) and distant fat (thoracic) pads. Consistent with fulvestrant penetrance through tumors, local delivery was more effective in reducing Ki-67 immediately adjacent to the tubing but maintained concentrations comparable to systemic therapy throughout the entire tumors. We further demonstrated that human fat cells readily take up fulvestrant and then transfer the drug to breast cancer cells. These findings support the use of local drug delivery through the human breast tissue and surrounding fatty tissue. Mammary tissues are rapidly cleared of fulvestrant upon removal of the drug-loaded tubing. This would allow the long term implantation of a drug delivery device designed to be emptied or refilled.

Local drug delivery is ideally suited in a setting of local disease or recurrence with minimal risk for systemic metastases with the goal of producing high concentrations without systemic application of the drug. Our data support the concept of a local silastic tubing device as a means to locally deliver an anti-estrogen in three major applications: early interventions for localized tumors, such as ductal carcinoma in situ (DCIS) or early stage breast cancer with low metastatic potential, prevention of breast cancer in women at higher risk due genetic predisposition, or used in concert with systemic therapy to provide a localized therapeutic boost. Overall, the use of implantable silastic tubing for local drug delivery represents a promising approach and introduces a potential paradigm shift in prevention and treatment of breast cancer.
Title: α-tocopherol succinate enhances the anti-tumor activity of pterostilbene against human breast cancer cells in vivo and in vitro

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Body: Background: The cancer preventive activity of vitamin E (α-tocopherol, α-TP) has been suggested by many epidemiologic studies. Our previous study showed that Tocopherol-Associated Protein (TAP), a major tocopherol–binding protein, is preferentially detected in normal breast tissues than in tumor regions among 93 clinical breast cancer patients, which postulate its tumor suppressive role in breast cells. Pterostilbene (3,5-dimethoxy-49-hydroxystilbene, PS) is a natural dimethylated analogue of Resveratrol found in different plants. Previous studies have shown that PS has the ability to induce apoptosis in a range of cancer cell lines. The purpose of our study was to assess the effect of TAP and Pterostilbene in tumor suppression of breast cancer cell.

Methods: Cell growth, proliferation, and viability were determined using the MTT assay. TAP protein expression was ablated in MDA-MB-231 breast cancer cell with nine independent siRNA sequences. MDA-MB-231 cells were transfected with pcDNA5 plasmids expressing the firefly luciferase gene. TAP protein expressions for each plasmid transfection were measured by western-blot analysis. Synergic effects of tumor growth and metastasis inhibitions in MDA-MB-231 xenograft animal model by PS and α-tocopherol treatment were evaluated.

Results: TAP served as a key molecular in responding to α-tocopheryl succinate (α-TOS) induced cell cycle regulation. PS has demonstrated the synergic decrease on breast cancer cell growth effects by the combination of α-TOS exposure. In animal model, treatment of PS and high level vitamin E diet not only induced breast tumor growth inhibition, but also suppressed tumor cell invasion by measuring circulation tumor cells in mouse blood.

Conclusions: Upon the evidences showed in this study, we suggest taking vitamin E and PS as supplements may provide both breast tumor preventative and therapeutic benefits in clinic.
Title: Towards breast cancer prevention through reduced breast density: Suppressive effects of tamoxifen on normal breast epithelial cells

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Body: Background: Breast density is positively associated with risk of breast cancer and the local microenvironment in the normal breast is known to significantly impact breast cancer initiation and further progression. The selective estrogen receptor modulator tamoxifen, with beneficial clinical effects on breast cancer recurrence, has also been shown to reduce mammographic density potentially explaining the primary preventive effects of tamoxifen. Still, important gaps persist regarding the cellular and molecular basis of how tamoxifen modifies breast density and subsequently breast cancer risk.

Purpose: To investigate the impact by tamoxifen on normal human breast epithelial cells (MCF-10A) with the additional ambition to mimic variations in breast density on the cellular level.

Experimental design: Effects of tamoxifen on MCF-10A cells at different cellular densities were evaluated \textit{in vitro}. Flow cytometry and the sulforhodamine-B assay were used to assess cell cycle distribution and proliferation. Cell adhesion to extracellular matrix proteins was analyzed using hexosaminidase assay. Modulation of integrin receptor levels was determined by Western immunoblotting.

Results: Tamoxifen exposure resulted in impaired cell cycle progression of human breast epithelial cells with a dose-dependent G\textsubscript{2}/M-phase arrest and reduced cell proliferation. Irrespective of tamoxifen treatment, the percent cellular adhesion to fibronectin and collagen type I by MCF-10A cells was significantly greater in high-density models compared to low-density models. Following tamoxifen treatment the adhesion was reduced in all density models. Consistent with the tamoxifen-induced lower adhesion, the integrin $\alpha$1 and $\beta$3 levels were reduced in a dose-dependent way.

Conclusion: These data support the hypothesis that tamoxifen affects the normal breast epithelium and its adhesion capacity, which may contribute to the clinically observed decrease in breast density and a potential reduced risk of breast tumor establishment.
**Title:** Participant-reported symptoms as predictors of long-term adherence of endocrine therapy in the International breast cancer intervention studies 2 (IBIS-2)

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**Body:** Background: Aromatase inhibitors (AIs) reduce the risk of breast cancer in women at increased risk and reduce recurrence in those with ductal carcinoma in situ (DCIS) (IBIS-2, MAP.3, NSABP B-33). The effectiveness of AIs depends on full adherence. We have previously reported adherence figures for the International Breast Cancer Intervention Studies 2 (IBIS-2) when 5 years of active treatment was not completed. Here, we assess reports of early symptoms on 5-year adherence with anastrozole in the prevention (versus placebo) and DCIS (versus tamoxifen) IBIS-2 after active treatment has been completed by all women.

**Methods:** In IBIS-2, 3864 postmenopausal women in the prevention study were randomised to placebo vs. anastrozole (1mg/day) and 2980 postmenopausal women with DCIS were randomised to tamoxifen (20mg/day) vs. anastrozole (1mg/day). Women were excluded from the analyses (n=491 [262 prevention; 229 DCIS]) due to breast cancer, death, major adverse events, or failure to initiate preventive therapy. Adherence (<4.5 years, ≥4.5 years) was calculated using the Kaplan-Meier method. The primary objective was to determine overall adherence to endocrine treatment in both studies separately. Secondary objectives were to estimate the effect of early symptoms (6 months visit) on adherence by study and by treatment arm separately.

**Results:** In the IBIS-2 prevention study (N=3615), overall adherence to treatment was 67.7% and was statistically not significantly different between anastrozole (66.5%) and placebo (69.0%) (OR=0.89 (0.78-1.03), P=0.11). Adherence was significantly lower regardless of treatment allocation for those who developed arthralgia (68.3% vs. 72.8%, P=0.007) or gynaecological symptoms (vaginal changes, irregular bleeding) (65.1% vs. 72.2%, P=0.007), but not for those who reported hot flushes (71.1% vs. 71.8%, P=0.92), compared with those who did not report these symptoms at 6 months. In the IBIS-2 DCIS study (N=2759), adherence to treatment was 70.1% overall (anastrozole (70.2%) or tamoxifen (70.0%) (OR=1.01 (0.86-1.19), P=0.92)). Women treated with anastrozole reported significantly more arthralgia (30.6% vs. 20.5%, P<0.001), but significantly fewer hot flushes (41.1% vs. 47.0%, P=0.002) and gynaecological symptoms (7.0% vs. 12.6%, P<0.001) compared with those on tamoxifen. However, none of these symptoms had an impact on adherence to either anastrozole or tamoxifen. In both studies, the majority of symptoms were of mild or moderate severity and we observed significant trends for lower adherence with increasing severity for all symptoms irrespective of allocated treatment arm.

**Conclusions:** In the IBIS-2 trials, we observed no significant differences in adherence between either anastrozole vs. placebo (prevention), or anastrozole vs. tamoxifen (DCIS). Significant associations between early symptoms and adherence were observed only in the prevention study, regardless of treatment allocation. Reporting symptoms in the first 6 months of preventive and adjuvant therapy is unlikely to explain non-adherence to medication. Further research is required to identify modifiable factors which may be altered by behavioural interventions to improve adherence.
2017 San Antonio Breast Cancer Symposium

Publication Number: P5-16-01

Title: Documenting and sharing breast cancer knowledge from National Cancer Institute designated comprehensive cancer centers (NCI-CCCs) with community oncologists

Maitri Kalra1, Meghan Karuturi2, Debu Tripathy2, Rachel Jankowitz3, Kelly McCann4, Adam Brufsky3, Sara Hurvitz4, Oliver Bogler2, Samir Housri6 and Nadine Housri5. 1Indiana University, Indianapolis, IN; 2MD Anderson Cancer Center, Houston, TX; 3University of Pittsburgh Medical Center, Pittsburgh, PA; 4University of California, Los Angeles, Los Angeles, CA; 5Yale University, New Haven, CT and 6themednet.org.

Body: Introduction: Tumor boards (TB) at National Cancer Institute Designated Comprehensive Cancer Centers (NCI-CCCs) are an important source of multidisciplinary education. Unfortunately, expert knowledge from NCI-CCCs is not systematically documented and made accessible to oncologists in the community. This represents a lost opportunity to capture and share clinical expertise that can impact patient care in community centers. Using an online oncologist-only social network, we sought to demonstrate the feasibility of systematically documenting expert insights from TBs and department conferences at NCI-CCCs in order to expand their reach and provide educational benefit to the greater oncology community.

Methods: A pilot program was developed at the University of Texas MD Anderson Cancer Center (MDACC) to design a process in which discussions at departmental breast cancer conferences would be distilled down to clinical questions and answers (Q&A) and posted on theMednet.org, an online social Q&A website of over 3,800 US oncologists. An educational breast cancer conference was selected during a site visit. A faculty member was selected to distil discussions about patient management from the selected conference into a question that addressed the clinical situation being discussed. After the question was posted, the oncologist leading the discussion answered the question on theMednet. The Q&A was then indexed and stored for easy search retrieval and disseminated in a weekly newsletter to all registered medical oncologists. A detailed manual was created to document operating procedures for implementation at additional institutions.

Results: After developing the process at MDACC, the program was expanded to 2 additional NCI-CCCs- University of Pittsburgh (UPMC) and UCLA. The educational breast cancer conferences selected varied by site and were the new patient planning conference at MDACC, tumor board at UPMC, and multidisciplinary clinic at UCLA. The most significant factor for success was involvement of one faculty member who regularly identified educational questions and additional faculty who posted their answers. Between December 2016 and May 2017, 17 answers to 17 questions were posted and shared with over 1,200 medical oncologists via an email newsletter. All questions were focused on topics not answered by NCCN or ASCO guidelines. The majority of questions focused on management decisions around chemotherapy and endocrine therapy. Answers were viewed by 339 oncologists at 260 institutions in 47 states. This included 190 community practices and 70 academic medical centers.

Conclusion: We developed a process of capturing and sharing expert knowledge at NCI-CCC breast cancer conferences on questions not answered by current guidelines. These discussions are otherwise not documented or shared outside of academic centers. By translating discussions into actionable Q&A on an online oncologist network, we made them easily accessible to oncologists at nearly 200 community practices. Future efforts will be aimed at implementing the program into the breast cancer programs at additional NCI-CCCs.
Title: Variance between experts and community practitioners in treatment of metastatic breast cancer

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Body: Background

New treatment options continue to improve outcomes for patients with metastatic breast cancer (MBC). However, clinicians' lack of clinical experience using new agents, a complex treatment landscape, and the broad treatment recommendations in available guidelines can make the choice of an optimal treatment for individual patients with MBC challenging. An online treatment decision support tool was developed to overcome these challenges and provide recommendations from multiple experts for specific MBC patient scenarios. Here we report data comparing expert treatment recommendations with the intended treatment indicated by clinicians using the tool.

Methods

In October 2016, 5 breast cancer experts provided treatment consultation for 492 unique MBC case scenarios based on a simplified set of variables: disease phenotype, previous systemic therapy, visceral crisis (yes/no), and rate of disease progression. These patient and disease characteristics along with expert treatment consultation were used to develop the treatment decision tool. Clinicians used drop-down menus to enter patient and disease factors along with their intended treatment plan. When completed, the experts' treatment recommendations for that specific patient case were shown to the clinicians, at which point the users were asked to indicate if the expert recommendations changed their planned treatment.

Results

From December 2016 through April 2017, 619 healthcare providers entered 1018 patient case scenarios in the online MBC tool representing the following phenotypes: HR+/HER2- (53%), HR-/HER2+ (10%), HR+/HER2+ (14%), and triple-negative breast cancer (23%). A comparison of expert and community oncologist treatment choices in select patient case scenarios with expert consensus is shown in the table. Among participating oncologists whose initial intended treatment of MBC differed from the experts, 51% indicated that they would change their choice of therapy.

Conclusions

MBC therapy continues to evolve with new agents having a large impact on how experts treat MBC. Data from the online MBC treatment decision support tool indicate variance in expert and oncologist treatment choices for many case scenarios. Moreover, consensus expert recommendations in this online tool changed the intended treatment plan of many using it and, therefore, can help optimize the care of patients with MBC. A detailed analysis of self-identified practice trends among those using the online tool, along with a comparison of expert and participating oncologist treatment choices for different MBC case scenarios, will be presented.

<table>
<thead>
<tr>
<th>MBC Case Scenario</th>
<th>Majority Consensus Recommendation Among Experts, %</th>
<th>Tool Cases Where Oncologist Intended Treatment Matched the Expert Consensus Recommendation, %</th>
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<tbody>
<tr>
<td>HR+/HER2- (no visceral crisis)</td>
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<tr>
<td>• De novo</td>
<td>Palbociclib + letrozole: 100</td>
<td>23</td>
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<tr>
<td>• Previous (neo)adjuvant AI Palbociclib + fulvestrant: 92</td>
<td>19</td>
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<td>• Previous palbociclib + letrozole Fulvestrant: 82</td>
<td>0</td>
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<tr>
<td>• Previous palbociclib + fulvestrant Everolimus + exemestane: 87</td>
<td>50</td>
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<tr>
<td>HR-/HER2+</td>
<td>THP: 100</td>
<td>68</td>
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<tr>
<td>De novo</td>
<td>T-DM1: 100</td>
<td>66</td>
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<tr>
<th>Triple-negative breast cancer</th>
<th>Combination CT: 91</th>
<th>39</th>
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<tr>
<td>Visceral crisis</td>
<td>Single-agent CT: 72</td>
<td>50</td>
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<tr>
<td>No visceral crisis, fast progression</td>
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AI, aromatase inhibitor; CT, chemotherapy.
Global Cancer Institute online breast tumor boards: A tool to facilitate multidisciplinary discussions in resource-limited settings

Jessica St. Louis¹ ², Maria Cecilia Espalter¹ ² ⁵, Joel Moreno¹ ² ⁴, Vahagn Hambardzumyan¹ ² ⁶ and Paul E Goss¹ ² ³.

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**Body: Background:** Multidisciplinary tumor boards (MTBs) are commonly practiced among specialists in high-income countries (HICs) to ensure evidence-based, concordant decisions for patient treatment. MTBs have also been shown to improve patient outcomes and quality of life. Cancer specialists in low-and middle-income countries (LMICs) have limited time and few opportunities to discuss breast cancer patient care with colleagues. The Global Cancer Institute (GCI) established online MTBs in 2012 to facilitate live telemedicine discussions of breast cancer case scenarios between specialists in LMICs and expert specialists in HICs. GCI MTBs aim to improve clinical knowledge and patterns of practice among specialists in LMICs with a low-cost, interactive, and educational tool.

**Methods:** In each monthly, hour-long MTB, three de-identified breast patient case scenarios are presented in English by specialists in LMICs for live discussion with a multidisciplinary expert panel of breast specialists based in the US. Discussions are held for each case scenario and provide an overview of evidence-based treatment, international and resource-stratified clinical guidelines, clinical trials, and best clinical practices for limited resource settings. After each MTB, links to clinical practice guidelines, clinical trials, and other resources are shared with MTB attendees. For educational purposes, each MTB is privately live-streamed online and uploaded to a private YouTube channel for viewing by cancer specialists and trainees worldwide.

**Results:** The GCI MTB program has interacted with over 500 participants from 44 hospitals in 25 LMIC countries across Latin America (LA), Eastern Europe, Africa, and Asia. 17 expert breast cancer specialists from 10 US cancer centers provide multidisciplinary guidance for each case.

To date, 142 breast cancer case scenarios have been presented. For breast MTBs, 83% of case scenarios were invasive ductal carcinomas. Common subtypes presented were ER/PR+ (62%), HER2+ (31%), and triple negative disease (29%). 60 cases (43%) involved management of advanced disease in resource-limited settings.

**Conclusions:** GCI MTBs are a low-cost educational tool for specialists in LMICs to improve patterns of clinical practice and engage in multidisciplinary discussions with colleagues. GCI continues to expand its MTB among cancer facilities in LMICs worldwide. GCI will pilot the implementation of a LA-based online MTB conducted in Spanish for wider participation of community and rural/remote breast cancer specialists in the region.
Title: Effectiveness of education program of WEBINAR focusing on treatment and nursing for metastatic breast cancer patients (MBC)

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Body: Backgrounds We organized WEBINAR focusing on treatment and nursing of MBC for the first time in Japan. The purpose of this study is to investigate the learning effect to the nurses who attended this program and to verify the effectiveness of this educational program. Material and Methods This educational program consists of 13 chapters and each chapter contains lecture and case studies of 15 to 20 minutes length. It includes pathophysiology and treatment of MBC and special nursing for special condition of MBC, i.e. breast ulceration and bone metastasis with severe pain. The target audience is nurses with more than three years of breast cancer nursing experience. Educational program has been developed in cooperation with Certified Nurses in Breast Cancer Nursing, Certified Nurse Specialists in Cancer Nursing, pharmacists and a doctor. Teaching material was composed of several slides with commentary. It was distributed on-demand for free from November 2016 for three months. Eighty-five nurses who gave consent to this investigation from 771 participants of this program were analyzed for the correlation of learning effect of this program with their background. Also, each program was assessed in terms of effectiveness. Twenty six questions (two questions each for one program) were asked via the internet before and after the program (one question 1 point). The average of total score before and after the program was analyzed. In addition, we analyzed individual characteristics affecting the total score before and after the program by a two way factorial analysis of variance. Furthermore, we assessed the rate of change of the participants number who have given right answers (calculation formula: (number of participants with correct answer after program - before program) / number of participants with correct answer before program). This study was approved in the ethics committees of Chiba University Graduate School of Nursing Graduate School. Results Median age was 42.0 years, and median period of breast cancer nursing experience was 4.7 years. Average total score before and after the program was 20.5 points and 23.0 points respectively. There was no statistical significant correlation between individual characteristic with learning effect. The most effective chapter was “Treatment strategy of MBC (rate of change: 54%)”, followed by “Nursing of HBOC (40%)” and “Medication of MBC (34%)”. On the other hand, several chapters on nursing for MBC have low change rate (7%). Conclusion The participants have deepened their understanding of pathophysiology, treatment and nursing on MBC through this program. It provided good opportunity to learn about MBC for many nurses, and improved their level of nursing. This work was supported by JSPS KAKENHI(Grant Number 16K20748.)
Title: Lobular breast cancer alliance - Advocates advancing research, screening, treatment and follow-up care for lobular breast disease

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Body: Introduction

Thirty patient advocates attended the First International Invasive Lobular Breast Cancer Symposium in 2016 at the University of Pittsburgh Cancer Center. The conference underscored that invasive lobular breast cancer (ILC) and other lobular pathologies are understudied. Specifically:

ILC is the sixth most prevalent cancer of women and the second most frequently diagnosed histological subtype of breast cancer impacting up to 34,000 patients a year in the US.

ILC is a molecularly distinct breast cancer with unique subtypes and variants with differences in presentation and behavior, including physical findings of thickening and a tendency to metastasize to unique locations.

While ILC is frequently associated with a good initial prognosis, recent analysis suggests that long-term outcomes of ILC may be worse than those stage-matched to ductal breast cancer.

Current imaging tools are less reliable for early detection of lobular disease and detection of distant recurrence.

Standard of care chemotherapy and endocrine therapies may have different effectiveness applied to ILC and IDC.

Challenges

Growing interest in ILC research requires improved methods to identify, communicate with and link patients with ILC to clinical trials and research. Advocates with advanced science training are needed as partners for research proposals and grant reviews.

Lobular breast cancer is under-represented in key meetings and literature. Encouraging opportunities to share ILC research as agenda topics and fostering collaborations between researchers, clinicians and advocates can accelerate progress and refine clinical practices for screening, treatment and follow-up.

Patients living with ILC lack a central on-line source of lobular breast cancer information and resources. This information gap is a barrier to help patients recognize signs of lobular breast cancer's unique presentation and metastatic behaviors.

Results

The Lobular Breast Cancer Alliance (LBCA) was formed by patient advocates who attended the First International ILC Symposium in response to advocate-identified opportunities to advance research, refine treatments and enhance patient education.

LBCA's mission is to bridge patients, clinicians and researchers to increase our knowledge of lobular breast disease and promote research that leads to advancements in prevention, diagnosis, treatment and patient follow-up care.

Conclusions

LBCA is driving an increased awareness of lobular breast cancer with specific goals:


Elevate lobular research and foster opportunities for researcher, advocate and clinician collaborations at prominent conferences and meetings.

Identify barriers to conduct research on ILC and metastasis and link patients and advocates to ILC tumor research and clinical trials.

Initiate peer-to-peer clinician outreach strategy through targeted literature, meetings and education services to share information on ILC presentation, metastatic patterns and screening and treatment challenges of patients with ILC.

Build partnerships with existing breast cancer organizations to integrate ILC into existing information resources and work on common goals.
Title: Patient advocates are partners in global breast research: Value is recognised by early advocate involvement in the PREvent ductal carcinoma in situ invasive overtreatment now (PRECISION) study and related trials

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Body: Background
Early introduction and international collaboration of patient advocates is essential in the investigation of safe treatment of DCIS. This diagnosis has increased since the introduction of screening. The treatment of low risk DCIS has been the subject of debate, controversy, anxiety and cost to women and health services globally. There has been much "public" as well as "professional" discussion leading to considerable distress. Some surgeons have concerns about carrying out unnecessary surgery on patients with low risk DCIS, but this is current best practice due to lack of credible evidence that monitoring can be safe. Some women feel their "lives were saved" whilst others feel "mutilated" by "unnecessary" surgery. These women cannot know the true harm or benefit of the treatment which they receive without evidence from clinical trials and biological/molecular research.

Aims
· To provide evidence of the value of patient involvement.
· To encourage recruitment in "difficult to recruit studies" by educating the public, patients and clinicians.
· To assist in the design of effective information tools.
· To increase liaison between advocates internationally to understand differences in culture, health services, and patient expectation.

Methods
The PRECISION team includes 2 patient advocates from each of the DCIS randomized trials (LORIS in the UK, LORD in the Netherlands and COMET in the US).
Our group will liaise with other groups involved in the wider efforts around DCIS. We will take part in media interviews and public debates to raise awareness and the need for evidence to change practice to reduce overtreatment of low risk DCIS. We will work with our researcher colleagues in each PRECISION work project, join the PRECISION steering group, and liaise together via regular calls. Importantly, we will encourage awareness amongst clinicians to emphasize that patients wish to be informed about available trials, and that not to do so is denying patient choice.

Results
An increase in interest and recruitment can be measured and the influence of early involvement of patient advocates can be demonstrated so that the model can be used in other trials. The biology is intricate, scientific and exciting but it is crucial that the outcome is available and understood by all women and their physicians worldwide. The results will be promoted by patient advocates through publications, social media and patient groups. Advocates will appear as co-authors on scientific publications.

CONCLUSIONS With the increase in international clinical trials, there is a need for further understanding of the differences in practice and patient need in different hospitals - as well as in different countries. We will show the value of our collaboration by demonstrating the results of patient advocate involvement in the PRECISION program.

* The PRECISION Team is a Cancer Research UK Grand Challenge Award 2017 winning team and will be jointly funded by Cancer Research UK and the Dutch Cancer Society.
2017 San Antonio Breast Cancer Symposium

Publication Number: P5-17-03

Title: Breast cancer in young women in Canada: A needs assessment

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Body: Objective: A Needs Assessment was conducted by Rethink Breast Cancer to assess age-related differences in experiences for breast cancer patients. This was the first national survey to identify the needs and current gaps in care for younger breast cancer patients. The report, published in March 2013, provided critical evidence-based information and benchmarks to stakeholders on the associated challenges. The following data supplements the main findings presented at the 2013 SABCS by providing a descriptive overview of the differences in breast cancer experiences between younger (≤45 years of age) and older (>45 years of age) women.

Methods: From June to October 2011, an online bilingual (English and French) quantitative survey was open to Canadian women who had a diagnosis of breast cancer (initial or recurrence) in the prior 6 years. The survey focused on their pre-diagnosis, diagnosis, treatment, and post-treatment experiences. 574 women responded to the survey: 372 (65%) aged ≤45 years and 202 aged >45 years. The differences in responses between the younger and older respondents was analyzed using the Pearson's Chi-Square test (α = 0.05).

Results: Finding a lump (64.3 vs 39.6%; p < .01) and experiencing pain or discomfort in the breast (24.5 vs 14.4%; p < .01) were significantly more likely to cause concerns in younger women. Older women were significantly more likely to be concerned by results from a screening test/mammogram (45.0 vs 8.1%; p < .01). It is plausible that younger women do not receive mammograms as often as their older counterparts. Of the 319 women that found a lump, self-examination (38.6%) was the most frequent method of discovery. However, this was significantly more likely among older rather than younger women (51.3 vs 34.3%; p = 0.02). Follow-up appointments for future reassessment were significantly more likely to be scheduled with younger rather than older women (15.1 vs 5.0%; p = 0.02). With respect to treatment, younger women were significantly more likely than older women to be recommended chemotherapy (81.7 vs 67.3%; p < .01), targeted therapy (21.0 vs 10.9%; p < .01), bilateral mastectomy (17.2 vs 8.9%; p < .01), breast reconstruction (30.9 vs 14.4%; p < .01), node dissection (37.9 vs 23.3%; p < .01), oophorectomy (10.8 vs 5.0%; p = 0.02), and hysterectomy (7.8 vs 2.0%; p < .01). Transitioning from regular to occasional monitoring by a healthcare team was reported to be very or somewhat difficult (59.1 vs 41.8%; p < .01) for younger instead of older women, while older women were significantly more likely to report little to no difficulty with this transition (58.2 vs 40.9%; p < .01).

Conclusions: The Needs Assessment demonstrated significant age-related differences in almost all aspects of breast cancer care, including during pre-diagnosis, treatment, and post-treatment. Differences such as greater recommendations for more aggressive treatments and difficulty in care transitions may lead to challenges being faced by younger women relative to their older counterparts. Tools such as checklists and guidelines may assist healthcare teams in meeting the needs of younger women. Future studies are warranted to assess the impact of such tools in helping improve patient education, advocacy, and support programs for this population.
Changing the DCIS conversation: Development of an alternative discourse by patient stakeholders in the COMET study

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The conversation about ductal carcinoma in situ (DCIS) is structured and influenced by traditional oncology values and beliefs, resulting in current standards of guideline concordant care (GCC). It is now widely known that DCIS is a heterogeneous condition requiring a tailored treatment approach based on characteristics of the specific lesion. Further, “low-risk” DCIS—defined as low grade, hormone-receptor positive condition—may be a subset with lower likelihood of progression to invasive breast cancer. Yet, most women diagnosed with any type of DCIS are encouraged to undergo the same immediate, aggressive treatments that women diagnosed with invasive breast cancer receive. Emerging evidence suggests that surgery may not increase overall survival compared to no surgery in many women diagnosed with low-risk DCIS.

The COMET (Comparison of Operative to Monitoring and Endocrine Therapy) study is a new randomized, prospective clinical trial funded by the Patient-Centered Outcomes Research Institute that aims to determine whether active surveillance is a safe and reasonable alternative to GCC for patients with low-risk DCIS. COMET also seeks to change the way patients, providers, and other key stakeholders view DCIS management options. Thus, COMET governance integrates patient collaborators into every aspect of planning and development via a Patient Leadership Team (PLT) and an independent external multi-stakeholder advisory board (SAB). The PLT is a fully integrated, yet autonomous part of the COMET study governance and is comprised of 4 patient advocates who meet independently and with COMET Principal Investigators (PIs). The SAB is a fully external and independent body of clinicians, content experts, payers, patients and patient advocates, and policymaker advisors.

To facilitate the cultural shift, the PLT developed communication materials with language and terminology that aims to communicate a diagnosis of DCIS without increasing unnecessary fear and anxiety that often accompanies a diagnosis of invasive cancer. In collaboration with study team PI's and the SAB, PLT also has developed content for a newly established website (www.DCISoptions.org), which includes patient-centered materials that aim to help newly diagnosed patients understand that a majority of DCIS is low-risk and that they have time to make informed decisions about their care.

The PLT contends that managing DCIS is as much a cultural phenomenon as a scientific one. As such, changing the conversation about DCIS between medical providers and patients is essential for generating a cultural shift in understanding the condition, correcting risk perception, and enabling improvement in patient experience. The novel approach of a stand-alone PLT that is also integrated throughout the trial's stakeholder structure ensures patient-centered involvement across all aspects of the study. The unique talents of each stakeholder group are leveraged in a unified effort to educate the DCIS community about low-risk DCIS, with the overarching goal of enabling a patient to choose the treatment approach that best reflects her risk profile and personal preferences, thus minimizing potential physical, emotional, and financial harms.
Title: Extended continuous vs intermittent adjuvant letrozole in postmenopausal women with lymph node-positive, early breast cancer (IBCSG 37-05/BIG 1-07 SOLE): Impact on patient-reported symptoms and quality of life

Karin Ribi,1 Weixiu Luo,1 Marco Colleoni,1 Per Karlsson,1 Jacquie Chirgwin,1 Stefan Aebi,1 Guy Jerusalem,1 Patrick Neven,1 Vincenzo Di Lauro,1 Henry L Gomez,1 Thomas Ruhstaller1, Estesham Abdi,1 Angelo Di Leo,1 Bettina Müller,1 Rudolf Maibach,1 Richard D Gelber1, Aron Goldhirsch1, Alan S Coates1, Meredith M Regan1 and Jürg Bernhard1.1International Breast Cancer Study Group, Breast International Group.

Body: Background: SOLE efficacy results presented at ASCO 2017 showed that extended intermittent vs continuous letrozole for 5 years did not improve disease-free survival in postmenopausal women who had received 4-6 years of adjuvant endocrine therapy for hormone-receptor positive (HR+), lymph-node positive breast cancer. Previous studies showed that the burden by symptoms related to endocrine therapy can be substantial. Even if symptoms improve during the treatment course, extending treatment implies continuation of symptoms. We compared differences in patient-reported symptoms (PRS) and quality of life (QoL) between extended continuous and intermittent letrozole over the first two years of trial treatment.

Methods: From Nov 2007 to Dec 2010, 956 postmenopausal women who were disease-free following 4-6 years of prior adjuvant endocrine therapy for HR+, node-positive breast cancer were enrolled in the QoL substudy of the randomized phase III trial SOLE at selected centers. Patients receive extended continuous letrozole (2.5 mg daily) for 5 years or intermittent letrozole, taken for the first 9 months of years 1-4, and 12 months in year 5. 955 patients completed the 18-item Breast Cancer Prevention Trial (BCPT) Symptom Scales and further symptom-specific and global QoL indicators at baseline, and at 6, 12, 18 and 24 months after randomization. Differences in change of PRS and QoL from baseline between the two administration schedules were tested at 12 and 24 months for 8 symptom scales, 4 additional symptom and 4 global QoL indicators using mixed models with repeated measures.

Results: Small changes in PRS and QoL scores were observed between baseline and 12 months after randomization, i.e. at the end of the first treatment-free interval in the intermittent arm. These changes showed a consistent pattern of greater worsening for patients receiving continuous compared to patients receiving intermittent letrozole. Patients receiving continuous letrozole reported a significantly greater worsening in vaginal problems (p<.02), musculoskeletal pain (p<.03), sleep disturbance (p<.01), physical wellbeing (p<.01) and mood (p<.03). At 24 months (after 2nd treatment-free interval) patients with intermittent letrozole reported a greater improvement in hot flushes (p<.03) than those with continuous letrozole. Changes in the other outcomes did not significantly differ between arms at 24 months.

Conclusion: Although changes in PRS and QoL were small, there was a consistent pattern favoring the intermittent arm. For several symptoms and global QoL indicators, significantly less worsening was observed with the intermittent administration, mainly during the first year of extended treatment, due to small improvements during the treatment-free interval. Froma QoL perspective, women who suffer from endocrine side-effects in the extended setting may benefit from an intermittent administration.
2017 San Antonio Breast Cancer Symposium

Publication Number: P5-18-02

Title: Nab-Paclitaxel-based therapy in the first line treatment of metastatic breast cancer (IBCSG 42-12/BIG 2-12 SNAP): Impact of different schedules on quality of life

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Body: Background: The randomized phase II SNAP trial assessed three alternative reduced maintenance chemotherapy regimens using nab-Paclitaxel after a short term induction phase at conventional doses as first line treatment in patients (pts) with metastatic breast cancer (MBC). For all three regimens median progression-free survival was greater than achieved with full dose docetaxel (historical reference). Symptom palliation and quality of life (QoL) are important when deciding on therapeutic agents and schedules in MBC pts.

Methods: Of the 258 pts with MBC enrolled from April 2013 to August 2015 in the SNAP trial, 255 were included in the QoL analysis. Pts were randomized to three arms, each receiving the same induction chemotherapy based on 3 cycles of nab-Paclitaxel 150 mg/m² dd 1, 8, 15 Q28, which was reduced to 125 mg/m² after a safety review. The schedules of nab-Paclitaxel in maintenance therapy differed in each arm: Arm A) 150 mg/m² dd 1,15 Q28; Arm B) 100 mg/m² dd 1,8,15 Q28; Arm C) 75 mg/m² dd 1,8,15,22 Q28. Pts completed a QoL form to assess global and symptom-specific indicators (range 0-100) at baseline, and at day 1 of every cycle for the first 12 cycles on treatment, or until treatment discontinuation. Changes in QoL scores during induction (day 1 cycle 4 − baseline) and maintenance (day 1 cycle 12 − day 1 cycle 4) therapy were summarized descriptively per arm. Treatment effects on changes in QoL during maintenance therapy were analyzed by repeated measurement models including timepoints (from day 1 of cycle 4 to day 1 of cycle 12), induction start dose, age, and treatment arms as covariates.

Results: During induction therapy, mean changes [SD] in hair loss (Arm A: −70.2 [41.9]; Arm B: −77.3 [34.5]; Arm C: −72.6 [32.8]), sensory neuropathy (Arm A: −19.0 [25.2]; Arm B: −20.6 [22.7]; Arm C: −18.8 [23.8]), and treatment burden (Arm A: −12.9 [33.4]; Arm B: −13.4 [33.5]; Arm C: −11.4 [34.8]) showed the most pronounced worsening. During maintenance therapy, scores for sensory neuropathy remained impaired without worsening. No significant differences in changes for sensory neuropathy or the other symptoms were seen between arms, except for hair loss, with pts in arm C (mean difference 10.91; 95% CI [0.35, 21.48]; p=0.04) and B (mean difference 18.55; 95% CI [7.52, 29.59]; p=0.001) reporting a greater improvement compared to those in arm A. Pts in arm C reported a significantly greater improvement in mood compared to arm A (mean difference 13.34; 95% CI [6.08, 20.60]; p<0.001) and arm B (mean difference 9.62; 95% CI [2.84, 16.40]; p=0.01).

Conclusion: The effectiveness of alternative maintenance chemotherapy schedules with reduced doses after a short term induction phase at conventional doses must be weighed against a substantial worsening in sensory neuropathy during induction therapy, and scores continuing to be impaired without worsening with prolonged administration. During maintenance therapy, improvements were seen in the perception of hair loss and in mood, particularly in Arm B and C, with a similar tendency seen for some other QoL domains. A more frequent administration of reduced dose chemotherapy agents is favorable with respect to QoL in this setting.
**Title:** Relationships among breast symmetry, appearance investment, and body image dissatisfaction in breast cancer patients undergoing reconstruction

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**Body:** Purpose: Reconstruction as part of treatment for breast cancer is aimed at mitigating body image concerns post-mastectomy. Although algorithms are increasingly being developed to objectively assess breast reconstruction outcomes, previous research has not evaluated associations between objectively quantified breast aesthetic appearance and patient-reported body image outcomes. Further, the role appearance investment plays in a patient's body image is not well understood. We examined the extent to which objectively quantified breast symmetry and appearance investment were associated with body image dissatisfaction in patients undergoing cancer-related breast reconstruction. Method: Breast cancer patients in different stages of reconstruction (n=190) completed self-report measures of appearance investment and body image dissatisfaction. Vertical extent and horizontal extent symmetry values, which are indicators of breast symmetry across the vertical axis, were calculated from clinical photographs. Associations among symmetry, appearance investment, body image dissatisfaction, and patient clinical factors were examined. Multiple regression was used to evaluate the association of symmetry and appearance investment with body image dissatisfaction. Results: Vertical extent symmetry, but not horizontal extent symmetry, was associated with body image dissatisfaction. Multiple regression results indicated that decreased vertical extent symmetry (\(\beta = -0.24\), \(p <0.01\)) and increased appearance investment (\(\beta = 0.37\), \(p <0.001\)) were significantly associated with greater body image dissatisfaction while controlling for clinical factors. Conclusions: Our findings suggest that quantitatively measured reconstruction outcomes and appearance investment both significantly contribute to an understanding of patient-reported body image satisfaction during breast reconstruction treatment. Future studies are encouraged to utilize algorithms assessing breast aesthetic appearance and to consider patient values as predictors of body image outcomes.

| Symmetry Predicting Body Image Dissatisfaction Controlling for Clinical Factors |
|-----------------------------|-----------------|
| **Body Image Dissatisfaction (\(\beta\))** |
| VE Symmetry                 | -0.24**         |
| Appearance investment (ASI-R) | 0.37***        |
| Clinical factors controlled for |
| BMI                         | -0.01           |
| Prior chemotherapy           | 0.19            |
| Prior radiation therapy      | 0.08            |
| Prior complications          | 0.06            |
| Reconstruction timing (ref: Immediate) |             |
| Delayed                      | 0.06            |
| Reconstruction type (ref: Implant) |         |
| Autologous                   | -0.01           |
| Mixed type                   | 0.09            |
| Reconstruction Stage (ref: Pre-surgery) |          |
| Intermediate                 | -0.06           |
| Final | -0.15 |

*p<.05, **p<.01, ***p<.001; ref = reference group; Note ASI-R = Appearance Schemas Inventory-Revised
Body: Introduction:
Supporting the emotional needs of women diagnosed with breast cancer is a recognized priority for cancer clinicians and a core component of high quality care and survivorship programs. We hypothesized that mental health professionals would benefit from an educational program directed to enhance their practical knowledge of breast cancer.

Methods:
We designed an innovative educational forum for mental health professionals to broaden their practical knowledge regarding the physical and psychosocial effects of breast cancer. Diverse mental health professionals working in the Bay Area were invited. The day-long forum consisted of presentations and interactive discussion led by breast cancer physicians, mental health providers and patient advocates. An evaluation survey was administered at the end of the program. Descriptive statistics were performed of categorical results and open response items were aggregated.

Results:
Of 40 local pre-registrants, 18 mental health professionals (8 social workers, 6 psychologists, 3 other mental health professionals) attended. 88.2% of participants worked in a medical-based practice; 64.7% worked in an oncology-based practice. The majority of participants agreed the forum was relevant to their practice (82.3%), was well-organized (88.2%), would improve their care of patients (76.5%), and that they would recommend it to others (76.5%). The overall rating of the forum was positive (94.0%).

Conclusion:
The format and content of this forum represents a multidisciplinary educational model to enhance mental health professionals' understanding of breast cancer in order to better serve this unique patient population. Breast cancer clinicians, advocates and psychotherapists generated much enthusiasm and broad insights about meeting the psycho-emotional needs of breast cancer patients. This model can be replicated and extended to a national audience and to other cancer subpopulations.
**2017 San Antonio Breast Cancer Symposium**

**Publication Number:** P5-19-01

**Title:** Impact of palbociclib plus letrozole on patient-reported general health status compared with letrozole alone in ER+/HER2- advanced/metastatic breast cancer

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**Body:** BACKGROUND: Palbociclib plus letrozole significantly improved progression-free survival (PFS) compared with letrozole plus placebo in treatment-naive postmenopausal patients with estrogen receptor-positive (ER+), human epidermal growth factor receptor 2-negative (HER2-) metastatic breast cancer (MBC) in the phase 3 PALOMA-2 trial. Here, we compare patient-reported general health status with extended (max 53 cycles) follow-up (data cut off May 31st, 2017) (Pfizer: NCT01740427).

**METHODS:** PALOMA-2 randomized patients 2:1 to palbociclib + letrozole (n=444) or placebo + letrozole (n=222). Patient-reported outcomes were assessed at baseline, day 1 of cycles 1, 2, and 3, and day 1 of every other cycle from cycle 5 until the end of treatment using the EuroQol 5-Dimension Questionnaire (EQ-5D). The EQ-5D is a standardized measure of health status that consists of a descriptive system comprising the following 5 dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression rated at 3 levels (no, some, or extreme problems) and a single index score for health status (ranges generally from 0 [dead] to 1 [full health]) calculated using a standard algorithm. In addition, a visual analog scale (VAS) measured self-rated health status from 0 (worst imaginable) to 100 (best imaginable). Repeated measures mixed-effects analyses were performed to compare overall index and VAS scores between treatments, controlling for baseline.

**RESULTS:** Completion rates at baseline were >95% in each group. The mean (SD) scores at baseline were comparable between palbociclib plus letrozole and letrozole alone for the VAS (71.3 [21.2] vs 72.3 [19.8]) and the EQ-5D index scores (0.70 [0.25]) vs (0.73 [0.21]). Median follow up was 38 months for palbociclib plus letrozole and 37 months for letrozole only. No statistically significant difference in overall change from baseline in general health status was observed between the treatment arms. The proportion of patients reporting the presence of a problem at baseline was similar for palbociclib plus letrozole and letrozole, respectively: mobility (39% vs 39%), self-care (12% vs 12%), usual activities (44% vs 39%), pain (69% vs 65%), and anxiety/depression (54% vs 54%). No statistically significant difference in overall mean EQ-5D index scores (0.73 vs. 0.71) was observed between the treatment arms.

**CONCLUSION:** Addition of palbociclib to letrozole maintained general health status and EQ-5D index scores in ER+ HER2- advanced/metastatic breast cancer with no statistically significant differences observed compared to letrozole alone.
Title: Factors that influence posttraumatic growth in patients with breast cancer: A longitudinal trial

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Body: Objectives:
The main focus of previous research into coping with breast cancer, have been mostly with regards to the negative impacts such as emotional stress, anxiety, depression or cognitive dysfunction. However, patients also report positive changes that have come about as a result of their struggle with cancer. This is referred to as posttraumatic growth (PTG). The aim of this study was to assess the presence of PTG in breast cancer patients at two measurements and to explore the association between personality traits (resilience, optimism, self-efficacy) and positive emotions on PTG.

Patients and Methods:
The study group comprised of 80 women (with a mean age of 53.6 years, SD=10.2) recently diagnosed with stage I to III breast cancer. They completed self-report measures of PTG, resilience, optimism, self-efficacy and positive emotions up to three weeks after diagnose (t1) and then following by an update six months later (t2). Sociodemographic characteristics, emotional distress, anxiety and depression were also recorded.

Results:
Planned comparison: Breast cancer patients showed an increase of PTG from t1 to t2 (p=.00035). Resilience, optimism, self-efficacy and positive emotions did not change in the total sample from t1 to t2. Emotional distress, anxiety and depression have significantly declined from t1 to t2 (anxiety p=.015, depression p=.029, emotional distress p=.000065). Moreover, we found a significant positive correlation between positive emotions to PTG (r=-.386, p=.0004) and optimism to PTG (r=.334, p=.002) at t2, respectively. Depression t2 correlated negatively to PTG at t2 (r=-.264, p=.018).

Explorative results: We also calculated the difference between PTG t2 and t1. We found 67.5% (n=54) of the patients with an increase of PTG and 32.5% (n=26) without or with negative change. In a subgroup analysis we noticed that patients without benefit of PTG showed a decline of resilience (p=.015), self-efficacy (p=.028) and positive emotions (p=.045) between t1 and t2. Furthermore, anxiety and depression did not improve from t1 to t2 in this group.

Conclusions: Positive emotions and optimism represent indicators of PTG in breast cancer patients. A decline of anxiety and depression is associated with a higher rate of PTG. Psycho-oncological strategies related to these factors may support PTG in breast cancer patients. Future studies are needed to search for effective interventions to support patients in PTG.
Title: Psychosocial impact of a multi-modality surveillance program for women at high-risk for breast cancer

Andrea L Amico¹, Raymond Fang¹, Akila Raoul¹, Kristen Wroblewski¹, Sarah Nielsen¹, Caroline Weipert¹, Hiroyuki Abe¹, Deepa Sheth¹, Iris Romero¹, Kirti Kulkarni¹, David Schacht¹, Linda Patrick-Miller⁴, Marion Verp¹, Angela R Bradbury², Fay Hlubocky¹ and Olufunmilayo Olopade¹. ¹The University of Chicago, Chicago, IL; ²University of Pennsylvania, Philadelphia, PA and ³Independent Contractor.

Body: Purpose: To evaluate the psychosocial impact of semi-annual dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) screening in women at high-risk for breast cancer.

Background: For women with BRCA1 and BRCA2 mutations and/or a personal or family history of breast cancer, annual breast MRI has shown improved sensitivity and cancer detection compared to mammography. However, MRI's heightened sensitivity may lead to increased: false positives requiring additional follow-up biopsy/imaging; iatrogenic risk; and psychosocial distress, which all may negatively impact women's overall health-related quality of life.

Methods: Between 2004 and 2016, we assembled a prospective cohort of high-risk women undergoing semi-annual DCE-MRI and annual mammography. We reviewed a subset of this group. Participants completed psychosocial assessments at baseline and 6-month visits using the following measures: coping (MBSS); state/trait anxiety (STAI-S/T); depression (BDI-II); risk perception; and mental health (SF-36). Participants were classified according to Monitor or Blunter coping style. Mixed-effects logistic regressions models examined effects of demographics on psychosocial changes over time.

Results: 295 women were recruited to the study; 44% of the study participants had pathogenic mutations in BRCA1 or BRCA2 genes. 232 of 295 enrolled participants (78.6%) completed psychosocial assessments. For the total population: median age 44y (range: 21-73), 71% ≥college/post-graduate education; 84% Caucasian; 8% African American; 2% Latino; 99% with health insurance; 72% annual income of $>60,000. One third of women had a personal cancer history. Participants were evenly split between baseline Monitoring and Blunting coping style (49% and 51%, respectively). No significant differences were found between demographics (age, race, income, mutation, cancer type, cancer history) or psychosocial factors (baseline trait anxiety (p =0.64), depression (p =0.65), SF36 global health (p=0.66). After adjusting for education, race, cancer history and coping, women with ≥$60,000 income had lower trait anxiety (p<0.000) and greater mental health (p<0.001) than those with <$60,000 income. Over time, change in trait anxiety varied by coping (p=0.0006): Blunters did not experience significant changes in trait anxiety (p=0.072) while Monitors had significant diminished trait anxiety over time (p<0.001). For depression, women with ≥$60,000 income and college educated had lower BDI-II depression (p<0.000). Yet, women with a cancer history had significantly greater BDI-II depression (p = 0.048). Mental health over time varied by race as non-whites had greater gains in mental health (p=0.001) over time than whites (p=0.03).

Conclusion: Semi-annual DCE-MRI did not cause a significantly elevated state anxiety or depression, nor was there a significant decline in mental health over time for groups regardless of cancer history and genetic mutation status. Coping style may have an impact on psychosocial outcomes for those undergoing heightened surveillance over time.
Title: Health-related quality of life during a phase 2 study of talazoparib in patients with advanced breast cancer and germline BRCA1/2 mutations (ABRAZO)

Sara A Hurvitz1, Nicholas C Turner2, Melinda L Telli3, Hope S Rugo4, Audrey Mailliez5, Johannes Ettl6, Eva-Maria Grischke7, Lida A Mina8, Judith Balmaña8, Peter A Fasching10, Cristina Tudor11, Ruben GW Quek11, Alison L Hannah11, Mark E Robson12 and Andrew M Wardley13. 1University of California Los Angeles Health, Santa Monica, CA; 2Royal Marsden Hospital, The Institute of Cancer Research, London, United Kingdom; 3Stanford University School of Medicine, Stanford, CA; 4University of California San Francisco Comprehensive Cancer Center, San Francisco, CA; 5Centre Oscar Lambret, Lille, France; 6Klinikum Rechts der Isar, Technische Universität München, Munich, Germany; 7Universität's-Frauenklinik Tubingen, Eberhard Karls University, Tubingen, Germany; 8Banner Health, Phoenix, AZ; 9Hospital Vall d'Hebron, Barcelona, Spain; 10University of Erlangen, Erlangen, Germany; 11Medivation, Inc. (Medivation was Acquired by Pfizer Inc., in September 2016), San Francisco, CA; 12Memorial Sloan-Kettering Cancer Center, New York, NY and 13The NIHR Cancer Research UK Christie Clinical Research Facility, Manchester, United Kingdom.

Body: Background: Talazoparib (TALA; 1 mg/d) was well tolerated and exhibited promising antitumor activity in ABRAZO, a 2-cohort, 2-stage, open-label phase 2 study (NCT02034916) in patients (pts) with locally advanced or metastatic breast cancer and BRCA1/2 mutations following platinum-based therapy (cohort 1 [C1]) or ≥3 platinum-free cytotoxic-based regimens (cohort 2 [C2]). This analysis evaluates health-related quality of life (QoL) for both cohorts.

Methods: QoL was assessed on day 1 (baseline) and every 6 weeks for the initial 24 weeks and every 12 weeks thereafter, or sooner if progression was clinically suspected, using the EORTC QLQ-C30 and its breast cancer module, QLQ-BR23. For all scales, results were summarized using descriptive statistics for each cohort and at each time point, based on Characters (max 3400 including title, body and table [including spaces]): 3363 No abbreviations in title; title sentence case; define acronyms; no figures Category: Psychosocial, QOL, and Educational Aspects – Other 2 observed values and changes from baseline (clinically meaningful defined as ≥10-point change from baseline). Time to deterioration (TTD; defined as ≥10-point decrease in global health status [GHS]/functional scales or increase in symptom scales) analyses using survival analysis methods were carried out on the GHS/functional scales of QLQ-C30 and symptom scales of QLQ-BR23.

Results: GHS was maintained from baseline across all time points for both C1 and C2 except at week 24 in C2, when a statistically significant but not clinically meaningful improvement in GHS was observed. In C1, statistically significant and clinically meaningful improvement was observed at specific time points in 4 functional scales (body image, week 6; sexual functioning, week 24; social functioning, week 36; and future perspective, weeks 6, 18, and 24) and in 3 symptom scales (dyspnea, week 24; insomnia, week 24; and breast symptoms, weeks 6 and 36). Statistically significant and clinically meaningful deterioration in C1 was observed in 2 functional scales (emotional functioning, week 12 and end of treatment, and role functioning, end of treatment) and in 1 symptom scale (fatigue, week 6). In C2, statistically significant and clinically meaningful improvement was observed at specific time points in 4 functional scales (role functioning, week 24; social functioning, week 24; sexual enjoyment, week 18; future perspective, weeks 6, 12, and 18) and in 5 symptom scales (nausea/vomiting, week 18; pain, weeks 12, 18, and 24; insomnia, week 24; breast symptoms, weeks 12 and 18; and arm symptoms, week 48). For C2, no statistically significant and clinically meaningful deterioration was observed for any functional or symptoms scales across all time points, except in the dyspnea symptom scale at week 18. For C1 and C2, the median (95% confidence interval) TTD of GHS was 2.8 (2.1-3.0) and 5.5 (4.2-5.7) months, respectively. The median TTD for all QLQ-C30 functional scales for C1 and C2 ranged 2.1-3.1 and 4.2-5.6 months, respectively; the median TTD for all QLQ-BR23 symptom scales ranged 2.6-4.0 and 4.2-5.6 months, respectively.

Conclusions: The QoL of TALA-treated patients during ABRAZO was maintained. QoL is being evaluated among atients with germline BRCA1/2 mutated advanced BC treated with TALA vs physician's choice chemotherapy in the phase 3 EMBRACA trial (NCT01945775).
Title: *SLIM for survival*: Introducing a community cancer center weight loss program

Patricia H Hardenbergh¹, Melaine Hendershott¹, Sarah Giovagnoli¹, Christine Hasselbach¹, Alexander Urquhart¹ and Renea R Nilsson¹. ¹Shaw Cancer Center, Edwards, CO.

**Body: Background:** In November 2014 The American Society of Clinical Oncology published an official position statement on obesity and cancer that asserts, “Significant efforts are needed to … develop and disseminate effective strategies to help patients with cancer initiate and maintain healthy lifestyle changes after a cancer diagnosis.” Mounting evidence shows that weight gain after a breast cancer diagnosis may have negative prognostic implications. Conversely, healthy body weight, regular physical activity, and healthful diets have been shown to have positive effects on prognosis including reduced comorbidities and reduced mortality. Community cancer centers are challenged to provide survivorship programs that adequately address this issue for breast cancer patients. **Method:** In Spring 2015 The Shaw Regional Cancer Center (SRCC) in Edwards, CO initiated *SLIM for Survival*, a targeted weight loss program with the goal of providing patients with specialized education, training, and support to help them lose weight and initiate healthy lifestyle changes. *SLIM for Survival* was created by a multi-disciplinary oncology-specialized team including the SRCC medical director, registered dietitian, exercise physiologist, physical therapist, and licensed clinical social worker. The program involves four retreat-style weekends at the SRCC cancer caring house occurring over the course of seven - nine weeks. Each weekend includes professionally-guided nutrition, exercise, and behavior change counseling groups that progressively build on prior weekends. The individual needs of each participant is assessed and incorporated into a personalized plan to help participants take what they learn, practice, and plan during the retreats into making changes at home. SRCC has facilitated four sessions serving a total of 35 participants including 19 breast cancer survivors who had a BMI $\geq 26$ or had gained 10 or more pounds since their cancer diagnosis. Program outcomes were assessed by comparing pre and post program measurements of body composition and Quality of Life (QoL; measured via the Functional Assessment of Cancer Therapy - General). **Results:** 18 out of the 19 breast cancer survivors finished the full program. Over the seven to nine weeks, participants lost 12.13 lbs, 6.5% of total body weight, 3.6% of body fat, and show a 2.1 point decrease in BMI on average. In addition, participants report a significant improvement in overall contentment with quality of life, t(17) = -3.69, p < .01. **Discussion:** *SLIM for Survival* demonstrates an effective intervention implemented at the community level to help breast cancer survivors lose weight and improve quality of life. Future directions will aim to better understand the specific behavior changes being made to diet, physical activity and behavior change skill use during the program. In addition, long-term outcomes and possible changes needed to assure maintenance of positive results will be explored.
Defining priorities for research: Interim results of the Canadian metastatic breast cancer priority setting partnership

Nancy Nixon¹, Christine Simmons², Julie Lemieux³ and Sunil Verma¹. ¹Tom Baker Cancer Centre, Calgary, AB, Canada; ²British Columbia Cancer Agency, Vancouver, BC, Canada and ³Chu de Quebec, Quebec, QC, Canada.

Body: Background: Research priorities are generally determined by funders and researchers without direct involvement and input from patients and caregivers. Certain disease areas have incorporated the patient voice to determine patient driven priorities. In this study, this approach was employed to better understand the needs and priorities of metastatic breast cancer patients and their caregivers.

Methods: This study was conducted using methodology outlined by the James Lind Alliance. A steering committee of patients, physicians, patient advocates, and allied health care professionals was assembled to oversee the research study. The initial survey collected unanswered research questions from patients, caregivers, and clinicians. Responses were collected and categorized by consensus of the steering committee. Here we present the results from the national survey.

Results: Between November 2016 and April 2017, 733 responses from 311 individuals were collected (62% patients, 11% physicians, 9% caregivers or relatives, 5% nurses/allied health professionals, 2% patient organization representatives, and 10% other). The main themes for key patient priorities are: 136 (19%) related to treatment and monitoring, 78 (11%) linked lifestyle and alternative therapy, 58 (8%) regarded tumour biology, 53 (7%) regarded psychosocial aspects, 46 (6%) to diagnosis, 35 (5%) to toxicity, 24 (3%) to prevention, and 17 (2%) to young or pre-menopausal population. Two hundred and eighty-six (39%) were considered out of scope. The most frequently identified priorities included the role of alternative therapies for improving survival, the role of immune therapy for treating metastatic breast cancer, and the potential for improving outcomes with early detection/surveillance with modern treatment and diagnostic modalities.

Conclusion: Patient derived research priorities in advanced breast cancer point to an improved understanding of alternative therapies, integration of immune therapy and a focus on early detection of relapse. These priorities should be addressed by the research community to meet the needs of our patients with advanced breast cancer.
Title: Prolonged progression-free survival (PFS) in advanced HER2+ metastatic breast cancer with or without brain metastases: A pooled analysis of tucatinib phase 1b studies

Erika Hamilton¹,², Rashmi Murthy³, Cristiano Ferrario⁴, Alison Conlin⁵, Ian Krop⁶, Carla Falkson⁷, Qamar Khan⁸, Marc Chamberlain⁹, Todd Gray⁹ and Virginia Borges¹⁰. ¹Sarah Cannon Research Institute, Nashville, TN; ²Tennessee Oncology, PLLC, Nashville, TN; ³The University of Texas MD Anderson Cancer Center, Houston, TX; ⁴Segal Cancer Centre – Jewish General Hospital, Montreal, QC, Canada; ⁵Providence Cancer Center, Eastside, Portland, OR; ⁶Dana-Farber Cancer Institute, Boston, MA; ⁷University of Alabama Comprehensive Cancer Center, Birmingham, AL; ⁸University of Kansas Medical Center, Westwood, KS; ⁹Cascadian Therapeutics, Inc., Seattle, WA and ¹⁰University of Colorado Cancer Center, Aurora, CO.

Body: Background: Advances in the treatment for HER2+ breast cancer using sequential HER2 targeted therapies has resulted in improvements in outcome. Nonetheless, treatment of HER2+ metastatic breast cancer (MBC) following 1st and 2nd line HER2 targeted therapies is therapeutically challenging. Currently there is no consensus regarding a single standard of care for such patients including those with brain metastases (BM). Tucatinib is a highly selective oral tyrosine kinase HER2 inhibitor that has shown promising results in the treatment of HER2+ MBC in patients both with and without BM.

Methods: Two Phase 1b studies of tucatinib were pooled to identify and characterize the subgroup of patients with extended PFS as defined by achieving twice the median PFS observed in the trials.

Results: 77 patients treated at the recommended Phase 2 dose (tucatinib 300 mg PO BID) were analyzed, 50 in Study 004 (tucatinib + T-DM1) and 27 in Study 005 (tucatinib +/- trastuzumab +/- capecitabine). Patients received a median of 3 prior therapies including a taxane, trastuzumab, pertuzumab, T-DM1 (Study 005 only), and lapatinib. Median PFS was 8.2 months in Study 004 and 7.8 months in the triplet cohort in Study 005. 15 patients (19.5%) demonstrated PFS >16 months, representing an extended PFS subgroup. Of these patients, 50% had baseline brain metastases, similar to the percentage of all patients entering the trial at baseline. No differences in the incidence of hormone receptor status (positive or negative), presence of visceral metastases, number of prior HER2 regimens, time from diagnosis (initial and metastatic) to study initiation, metastatic disease burden at study entry (LDH, number of target and nontarget lesions, sum of diameters of target lesions) or age were seen when comparing patients with or without extended PFS.

Conclusions: Nearly 20% of patients treated with tucatinib combinations for late stage MBC demonstrate prolonged PFS defined as > 16 months, representing a significant subgroup of patients who have extended disease control. Patient characteristics such as hormone receptor status, presence of visceral disease, burden of disease and age did not predict for extended PFS on tucatinib. While the presence of BM has historically negatively impacted PFS in clinical trials, baseline BM did not differentiate patients who achieved extended PFS. Further molecular characterization of the extended PFS cohort could help to identify this patient group. These data, demonstrating a significant subgroup of patients with extended PFS, further support the use of tucatinib in the accruing randomized HER2CLIMB trial.
Title: HER2 mutations detected by ctDNA in ER+/HER2- metastatic breast cancer patients: Incidence and impact on clinical outcomes

Arielle Medford¹, Dejan Juric¹, Andrzej Niemierko¹, Giuliana Malvarosa¹, Hannah Park¹, Maria Shellock¹, Laura Spring¹, Beverly Moy¹, Steven Isakoff¹, Leif Ellisen¹ and Aditya Bardia¹. ¹Massachusetts General Hospital, Boston, MA.

Body: Background: While the human epidermal growth factor receptor 2 (HER2) gene has long been linked with the pathogenesis and prognosis of breast cancer, its significance has been recognized only when the receptor has been amplified. However, rare, but actionable, somatic mutations in HER2, without HER2 gene amplification, have been described in breast cancer based on molecular analysis of metastatic specimens (frequency ~ 1%). Little is known about the incidence based on blood-based genotyping assays, as well as impact of HER2 mutations on clinical outcomes in patients with ER+/HER2- metastatic breast cancer (MBC).

Methods: We evaluated the presence of HER2 mutations based on routine tissue and blood-based genotyping assays, ordered by treating oncologists at our institution, for patients with estrogen receptor positive (ER+)/HER2 negative MBC. The tissue analysis was based on SNAPSHOT-NGS, an anchored multiplex polymerase chain reaction assay that detects single nucleotide variants (SNV) and insertions/deletions (indel), and the blood-based genotyping analysis was based on circulating tumor DNA (ctDNA) detection using the Guardant 360 panel, a next-generation sequencing (NGS) assay capable of detecting mutations with comparable sensitivity to SNAPSHOT. Patients with acquired HER2 mutations were identified, and multivariate analysis was performed to evaluate the hazard ratio (HR) for the association between HER2 mutations and progression free survival (PFS), adjusting for age and number of prior therapies.

Results: Among the ER+/HER2- MBC patients (N=118), 11% (N= 13) were found to have acquired HER2 mutation by ctDNA analysis, but no HER2 mutations were identified in any of patients based on tissue-based molecular analysis of archival specimens. Among patients with HER2 mutant, ER+/HER2- MBC, the median age at metastatic diagnosis was 57.34 (range 51.5-67.1) years, 7.7% had de-novo metastatic disease, and 30.8% had prior CDK 4/6 inhibitor therapy. In terms of outcomes, in the multivariate model, patients with HER2 mutant breast cancer had similar PFS when treated with endocrine and targeted therapy combination (HR = 0.24; p = 0.21), and trended towards worse PFS with chemotherapy (HR = 2.69; p = 0.06), as compared to non-HER2 mutant group, albeit duration of follow up was limited (median duration = 6.7 months). Of note, most of the detected HER2 mutations were activating or deleterious, but not all were clonal. Updated outcome data, including overall survival, will be presented at the meeting.

Conclusions: A much larger subset of patients with ER+/HER2- MBC have HER2 mutations detectable by ctDNA, but not by tissue, which highlights the need for blood-based biomarker monitoring for identification of actionable mutations, as well as the potential clinical utility in development of genotype driven trials for patients with HER2 mutant, ER+/HER2- MBC.
Impact of prior adjuvant trastuzumab (aT) on clinical characteristics, patterns of recurrence and outcome in 2863 patients with Her2 positive (HER2+) metastatic breast cancer (MBC): Results from the French ESME UNICANCER program

Mahasti Saghatchian1, Matthieu Carton2, Irwin Piot3, David Pérol4, Barbara Pistilli1, Etienne Brain2, Amal Ghouadni1, Francesco Ricci1, Laurence Vanlemmens4, Agnes Loeb3, Christelle Levy5, Anthony Goncalves6, Florence Dalenc1, Claudia Lefeuuvre-Plesse8, Mario Campone8, Anne Jaffre10, Sophie Gourgou11, Christian Cailliot3, Mathieu Robain3 and Veronique Dieras4. 1Institut Gustave Roussy, Villejuif, France; 2Institut Curie; 3Unicancer; 4Centre Oscar Lambret; 5Centre François Baclesse; 6Institut Paoli-Calmettes; 7Institut Claudius Regaud; 8Centre Eugène Marquis; 9Institut de Cancérologie de l'Ouest - René Gauducheau; 10Institut Bergonie and 11ICM-Montpellier Cancer Institute.

Body: Background: The management of HER2+ BC has changed dramatically with the introduction and widespread use of HER2-targeted therapies, especially in the adjuvant setting. However, there is relatively limited real-world information on the impact of adjuvant Trastuzumab (aT) on patterns of recurrence and outcome of HER2+ MBC.

Methods: In 2014, the 18 French Cancer Centers launched the Epidemiological Strategy and Medical Economics (ESME) program to provide real-world data on MBC patients (pts). All pts who started a 1st-line treatment for MBC between 01-Jan-2008 and 31-Dec-2014 were included. We examined clinical characteristics and outcomes (overall survival [OS] and time to next treatment [TNT]) in patients with HER2+ MBC pretreated with trastuzumab in the adjuvant setting (aT) compared with trastuzumab-naïve patients (nT) and patients with de novo HER2+ MBC (dn). Multivariate analyses adjusted for baseline demographic, prognostic factors and year of diagnosis (prior or after 2005, when aT was approved and widely administered in France for early HER2+ breast cancer).

Results: Among the 15170 pts of the ESME database, 2863 (19%) were HER2+: 1093 pts (38%) had de novo and 1765 pts (62%) recurrent MBC; 63% were Hormone Receptor (HR) +; 54%, 25% and 21% had respectively 1, 2, or > 2 metastatic sites (68% visceral and 12% brain). Median time to 1st metastasis was 43.4 months (m) (95% CI: 24.6-84.4): 54 m in HR+ and 30 m in HR-. Among pts with recurrent MBC, 55% (995) had received aT. As 1st-line therapy for MBC, 90 % of pts received HER2-targeted agents (73% T-based). With a median follow-up of 46 m, median OS is 45 m (95% CI: 42.5-48). OS is significantly higher in de novo compared to recurrent MBC: 54 m (95% CI: 50.2-60.4) vs. 38.4 m (95% CI: 36.7-41.9), (p < 0.0001). Among pts with recurrent cancers, median OS is inferior in pts who had received aT, as compared to those who had not: 33.4 m (95% CI: 29.6-36.7) vs. 49.5 m (95% CI: 44.3-56.8), (p < 0.0001). Statistically significant differences persist after adjustment for age at MBC, disease-free interval, metastatic sites and RH status in the multivariate model (HR=1.45, 95% CI: 1.26-1.67) but not after adjustment for year of diagnosis (prior or after 2005) (HR=0.90, 95% CI: 0.70-1.15).

Conclusions: These large-scale real-world data in patients with HER2+ MBC provide evidence that the survival outcome remain similar in patients with failure of adjuvant trastuzumab compared with trastuzumab-naïve patients after adjustment for year of diagnosis. De novo HER2+ MBC pts have the best outcomes. Data on clinical characteristics of metastasis and time to next treatment for the three subgroups will be presented at the meeting.
2017 San Antonio Breast Cancer Symposium

Publication Number: P5-20-04

Title: Safety of adjuvant treatment with pertuzumab plus trastuzumab after neoadjuvant anthracycline-based chemotherapy in patients with HER2-positive localized breast cancer: Updated results from the BERENICE study

Chau Dang1, Michael S Ewer2, Suzette Delaloge3, Jean-Marc Ferrero4, Mark Verrill5, Ramon Colomer6, Cláudia Vieira7, Luis de la Cruz Merino8, Jennifer Lucas9, Theresa L Werner10, Hannah Douthwaite11, Denise Bradley11, Maeve Waldron-Lynch11, Jennifer Eng-Wong12 and Sandra M Swain13. 1Memorial Sloan-Kettering Cancer Center, New York, NY; 2The University of Texas MD Anderson Cancer Center, Houston, TX; 3Institut Gustave Roussy, Paris, France; 4Centre Antoine Lacassagne, Nice, France; 5Northern Centre for Cancer Care, Newcastle upon Tyne, United Kingdom; 6Hospital Universitario La Princesa, Madrid, Spain; 7Instituto Português de Oncologia Francisco Gentil (IPOFG), Porto, Portugal; 8Hospital Universitario Virgen Macarena, Seville, Spain; 9Marin Cancer Care, Greenbrae, CA; 10Huntsman Cancer Institute, University of Utah, Salt Lake City, UT; 11Roche Products Ltd, Welwyn Garden City, United Kingdom; 12Genentech Inc, South San Francisco, CA and 13Georgetown University Medical Center, Lombardi Comprehensive Cancer Center, Washington, DC.

Body: Background Anti-HER2 therapies are associated with a risk of cardiac toxicity, particularly as part of anthracycline-based regimens. BERENICE (NCT02132949), a nonrandomized, Phase 2 cardiac safety study showed neoadjuvant treatment with pertuzumab (P) + trastuzumab (H) and 2 common anthracycline–taxane-based regimens had a safety profile consistent with prior studies of P+H, and was associated with high pathologic complete response rates. Here we report safety data from the P+H adjuvant treatment period (AP).

Methods Patients (pts) with centrally confirmed, localized HER2-positive breast cancer and normal cardiac function received 4 q2w dose-dense doxorubicin and cyclophosphamide cycles (60/600mg/m²) then 12 qw paclitaxel injections (80mg/m²; Cohort A), or 4 q3w fluorouracil/epirubicin/cyclophosphamide cycles (500/100/600 mg/m²) then 4 q3w docetaxel cycles (75mg/m², up to 100mg/m²; Cohort B). In both cohorts, 4 q3w cycles of P (loading:840mg; maintenance:420mg) + H (loading:8mg/kg; maintenance:6mg/kg) were started with taxane therapy and continued in the adjuvant setting (for up to 13 cycles to complete 1 year of treatment). Surgery was scheduled after 8 cycles of preoperative therapy. Primary endpoints were incidence of New York Heart Association (NYHA) Class III/IV heart failure and incidence of left ventricular ejection fraction (LVEF) declines (≥10%-points from baseline to <50%; asymptomatic and symptomatic events) assessed by ECHO/MUGA. Confirmed LVEF declines were defined as significant LVEF declines at 2 consecutive visits.

Results In total, 397 pts received ≥1 dose of study medication and were included in the overall treatment period (OTP) safety analysis. Of these, 371 (Cohort A:181; Cohort B:190) pts entered the AP and were included in the AP safety analysis. Mean (SD) number of AP treatment cycles of P and H were 12.3 (2.0) in Cohort A and 12.3 (2.2) in Cohort B. In the AP, incidence of heart failure was minimal (0.5%) and confirmed LVEF decline incidence was low (Table 1).

Table 1: Cardiac AE

<table>
<thead>
<tr>
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<th>Cohort A</th>
<th>Cohort B</th>
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<tbody>
<tr>
<td></td>
<td>OTP n=199</td>
<td>AP n=181</td>
</tr>
<tr>
<td>NYHA Class III/IV heart failure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Events, n</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Pts with event, n (%)</td>
<td>3(1.5)</td>
<td>0</td>
</tr>
<tr>
<td>LVEF decline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Events, n</td>
<td>36</td>
<td>22</td>
</tr>
<tr>
<td>Pts with LVEF decline, n (%)</td>
<td>21(10.6)</td>
<td>14(7.7)</td>
</tr>
<tr>
<td>Pts with confirmed LVEF decline, n (%)</td>
<td>7(3.5)</td>
<td>5(2.8)</td>
</tr>
</tbody>
</table>

General adverse events (AEs) are shown in Table 2; 26 (14.4%) pts in Cohort A and 45 (23.7%) in Cohort B had diarrhea AEs
Table 2: General AE

<table>
<thead>
<tr>
<th></th>
<th>Cohort A</th>
<th>Cohort B</th>
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<tbody>
<tr>
<td></td>
<td>OTP n=199</td>
<td>AP n=181</td>
</tr>
<tr>
<td>Pts, n (%)</td>
<td>198 (99.5)</td>
<td>171 (94.5)</td>
</tr>
<tr>
<td>Any AE</td>
<td>198 (99.5)</td>
<td>171 (94.5)</td>
</tr>
<tr>
<td>Grade ≥3 AE</td>
<td>109 (54.8)</td>
<td>23 (12.7)</td>
</tr>
<tr>
<td>Serious AE</td>
<td>54 (27.1)</td>
<td>15 (8.3)</td>
</tr>
<tr>
<td>AE leading to P or H discontinuation</td>
<td>19 (9.5)</td>
<td>9 (5.0)</td>
</tr>
</tbody>
</table>

**Conclusion** P+H in the adjuvant setting, following P+H with anthracycline-based regimens in the neoadjuvant setting, are associated with low incidence of cardiac AEs. Cardiac safety results for P+H in the AP and OTP of BERENICE were consistent with results from prior studies evaluating adjuvant treatment with single-agent H, suggesting the addition of P to H in the adjuvant setting does not increase cardiac toxicity.
2017 San Antonio Breast Cancer Symposium

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Title: Impact of type of (neo)adjuvant systemic therapy (AdjTx) and total exposure to trastuzumab (TET) on long-term outcome of HER2-positive (HER2+) early stage breast cancer (ESBrCa)

Giuseppe Gullo¹,², Naomi Walsh³, David Fennelly¹, Janice Walshe¹, Kate O'Mahony¹, Nuno Silva⁴, Josephine Ballot⁵, Giulio Calzaferri⁵, Cecily Quinn¹, Deirdre McDonnell⁵ and John Crown¹. ¹St Vincent's University Hospital, Dublin, Ireland; ²University College Dublin - School of Medicine, Dublin, Ireland; ³National Institute for Cellular Biotechnology, Dublin, Ireland; ⁴St Vincent's Private Hospital, Dublin, Ireland and ⁵Cancer Clinical Research Trust, Dublin, Ireland.

Body: Background
Trastuzumab (T) administered for 12 months (mos) as part of a taxane (Tax)– or Tax+anthracycline (Anthra)–based AdjTx is the standard of care as (neo)AdjTx of HER2+ ESBrCa. Several prospective randomized trials have investigated a shorter duration of Adj T (i.e. 9 weeks or 6 mos) compared to standard 12 mos of T. However, the results have not been conclusive so far. The impact of administering non-Tax/non-Anthra-based AdjTx and single-agent T on long-term outcome of HER2+ ESBrCa is not fully known.

Methods
We conducted a retrospective analysis on a prospectively maintained departmental database of all patients (pts) with Stage I-III HER2+ ESBrCa treated with at least one dose of (neo)Adj T. Pre-planned duration of T was 12 mos for all pts. TTE was defined as the interval in weeks between the first and the last dose of T. In order to ensure that most pts had a minimum FU of 3 yrs we included all pts who received 1st T before March 31st 2014. The database was locked for outcome analyses on March 31st 2017.

Results
506 pts treated between October 2001 and March 2014 were included in the study. Main pts characteristics: median age: 55 years (range: 26-85), oestrogen (ER) and/or progesterone (PR) receptors positive: 321 (63%), axillary lymph nodes positive: 266 (52%), Adj T: 386 (76%), neoAdj T: 120 (24%), Tax- and Tax/Anthra–based AdjTx: 457 (90%), non-Tax/non-Anthra AdjTx and single-agent T (without chemotherapy): 49 (10%). Median FU is 73.3 months (range: 1.4-176.3). In the overall population, DFS and OS rates are 83% and 91%, respectively. Pts treated with non-Tax/non-Anthra AdjTx had a significantly higher risk of BrCa relapse [DFS: HR 3.54 (95%CI:1.24 to 10.06, p=0.018)], and death [OS: HR 2.73 (95%CI:0.63 to 11.77 p=0.176)] compared to those treated with Tax–based AdjTx (e.g. TCH [docetaxel/carboplatin/T]). Pts who received single-agent T also had highly significantly worse DFS [HR 4.21 (95%CI:2.18 to 8.38, p<0.0001)] and OS [HR 6.75 (95%CI:3.13 to 14.6 p=<0.0001)] compared to those treated with Tax-based AdjTx. When adjusted for age (<55 vs >55 yrs), the detrimental impact of type of AdjTx remained highly statistically significant (p<0.0001). Patients with TTE<24 weeks had a highly significantly worse DFS [HR 4.7 (95%CI:2.34-9.47, p<0.0001)] and OS [HR 5.36 (95%CI:2.39-12.01, p<0.0001)] compared to pts with TTE>24 weeks. In most cases, shorter duration of T was due to reduction in LVEF or patients refusal. In the multivariate model, positive lymph nodes, type of (neo)AdjTx and TET (<24 weeks vs >24 weeks) remained all significant and independent variables associated with worse DFS and OS.

Conclusions
Our mature results indicate that the administration of non-Tax/non-Anthra-based AdjTx and single-agent T is associated with a significant increase in the risk of disease relapse and death and should not be considered as therapeutic options for pts with HER2+ ESBrCa. The administration of T for <24weeks irrespective of the type of AdjTx is also associated with significantly worse outcome.
Phase 1 dose escalation with ZW25, a HER2-targeted bispecific antibody, in patients (pts) with HER2-high breast cancer (BC)

Erika Hamilton¹, Funda Meric-Bernstam², Jeffrey Infante¹, Rashmi Murthy², Amita Patnaik², Sarina A Piha-Paul², Anthony Tolcher², Diana Hausman¹, Nels Royer¹ and Murali Beeram³. ¹Sarah Cannon Research Institute and Tennessee Oncology, Nashville, TN; ²The University of Texas MD Anderson Cancer Center, Houston, TX; ³South Texas Accelerated Research Therapeutics (START), San Antonio, TX and ⁴Zymeworks Inc., Vancouver, BC.

Body: Background: ZW25, a novel IgG1-like bispecific antibody, targets the same domains of HER2 as trastuzumab (T) and pertuzumab (P). In preclinical studies, ZW25 demonstrated increased tumor cell binding density and internalization relative to T and activity in T-resistant cell lines as well as models of HER2-low to high cancers. Initial dose escalation data demonstrated that once-weekly ZW25 was well tolerated at all doses evaluated and associated with single-agent anti-tumor activity in pts with heavily pre-treated (tx) HER2-expressing cancers.

Methods: 3+3 dose escalation of ZW25 given weekly (QW; 5, 10 or 15 mg/kg) or biweekly (Q2W; 20 mg/kg) in 4-week cycles. Eligibility included HER2 IHC 1, 2 or 3+ or FISH+ BC, progression after T, P and T-DM1, and measurable or non-measurable disease per RECIST 1.1. Active brain metastases were excluded. Baseline brain MRI was performed in QW cohorts only if pts had prior history (hx) of CNS mets, and in all Q2W pts regardless of hx. Assessments included AEs, LVEF, immunogenicity, PK and tumor response every 2 cycles.

Results: 8 pts with HER2-high BC were tx with ZW25 QW at 5 (n=2), 10 (n=2) or 15 mg/kg (n=4); 20 mg/kg Q2W is enrolling. 5/8 pts were HR+; 7 had measurable disease, 6 visceral disease, and 3 stable CNS disease. Median years since initial dx was 6 (range 5-16). Prior tx included T and T-DM1 (n=8); P (n= 6), and lapatinib (n=5). Median number of prior HER2-targeted regimens for metastatic disease was 6 (range 3-7) and non-hormonal HER2 regimens was 5 (range 3-7). ZW25 was well tolerated with no DLTs or decreases in cardiac function. Most common related AEs (all Gr 1 or 2) were diarrhea (n=4), infusion reaction (IR) (n=4) and vomiting (n=3). All IRs occurred only with 1st dose. There were no treatment-related SAEs. Related Gr 3 AEs (hypophosphatemia, fatigue and arthralgia) were reported in 1 pt (10 mg/kg).

At data cut-off, pts had received 2-10 cycles of treatment, with 3 pts active. Best overall response was 2 PR (10 mg/kg), 3 SD (1 at 5 mg/kg, 2 at 15 mg/kg), and 3 PD (1 at 5 mg/kg, 2 at 15 mg/kg) for a disease control rate of 63%. Decreases in target lesions were seen in 6/7 patients with at least one tumor re-staging. One pt with SD (5 mg/kg; active on study) had an 8% decrease after C2, and 29% decrease after C8. One PR pt with prior hx of brain mets had a 33% decrease after C2 and 44% decrease after C4, although was found to have new leptomeningeal disease (LMD) at that time. Two additional pts with systemic SD (15 mg/kg; no prior hx of CNS mets) were also considered to have PD due to symptomatic brain mets. One of these pts remains on study after receiving stereotactic radiotherapy.

Conclusions: ZW25 was associated with single-agent anti-tumor activity and systemic disease control in HER2-high BC pts after a median of 6 prior HER2-targeted regimens for metastatic disease. Systemic disease control was maintained despite PD due to brain mets or LMD. The presence of CNS disease in an unscreened population is consistent with the biology of late-stage HER2-high BC. The activity and tolerability of ZW25 support further evaluation as a single agent and in combination including with CNS-directed therapies in early and late lines of treatment for HER2-expressing BC.
Title: A phase Ib dose-finding study of subcutaneous pertuzumab in combination with subcutaneous trastuzumab in healthy male volunteers and female patients with early breast cancer

Whitney P Kirschbrown¹, Chris Wynne², Matts Kagedal¹, Russ Wada³, Hanbin Li³, Ihsan Nijem¹, Tanja Badovinac Crnjevic⁴, Sarah Heeson⁵, Jennifer Eng-Wong¹ and Amit Garg¹. ¹Genentech, Inc., South San Francisco, CA; ²Christchurch Clinical Studies Trust, Christchurch, New Zealand; ³Certara, Menlo Park, CA; ⁴F. Hoffmann-La Roche Ltd, Basel, Switzerland and ⁵Roche Products Ltd, Welwyn Garden City, United Kingdom.

Body: Background:
A fixed-dose combination (FDC) of subcutaneous (SC) pertuzumab (F. Hoffmann-La Roche Ltd, Basel, Switzerland) + SC trastuzumab (F. Hoffmann-La Roche Ltd) is being developed to reduce the treatment burden on patients while improving treatment facility efficiency. This phase Ib dose-finding study (NCT02738970) aimed to identify the SC pertuzumab dose that is comparable to the intravenous (IV) dose, based on serum trough concentrations (C_{trough}) and area under the concentration–time curve (AUC) when administered with or without SC trastuzumab.

Methods:
This two-part study consisted of SC pertuzumab dose determination in healthy male volunteers (HMVs) (Part 1) and a subsequent SC pertuzumab dose confirmation in patients with early breast cancer (EBC) (Part 2). Part 1 of the study was comprised of 48 HMVs who received various SC pertuzumab doses (400–1200 mg) or the standard IV dose (420 mg), administered alone or co-mixed with SC trastuzumab 600 mg. Non-compartmental and statistical methods were used to test the pharmacokinetic (PK) interaction between SC pertuzumab and SC trastuzumab when administered with recombinant human hyaluronidase, a permeation enhancer. Two population PK (popPK) models were built to estimate PK parameters and PK variability. Model 1 used IV/SC PK data from Part 1 of the current study only. Model 2 used Part 1 SC PK data and PK parameters from the published IV pertuzumab popPK model (Garg A, et al. Cancer Chemother Pharmacol 2014; 74: 819–829). Each popPK model was used to simulate 400 phase III clinical trials. Per simulated trial, the geometric mean ratio (GMR) of Cycle 8 C_{trough} at steady state and AUC at steady state for SC/IV were calculated. The percentage of trials with the 5th percentile confidence interval of the GMR above 0.8 was tabulated.

Results:
In Part 1 of the study, there was no impact on pertuzumab or trastuzumab PK from co-mixing SC trastuzumab with SC pertuzumab. The absolute bioavailability of SC pertuzumab in HMVs was approximately 70–80%, with a median time to reach maximum concentrations of 4–5 days. Clinical trial simulations indicated that an SC pertuzumab dose of 600 mg will achieve the target C_{trough} and AUC SC/IV GMRs > 99% of the time. Results were consistent between the models. Safety data supported the selection of an SC pertuzumab maintenance dose of 600 mg. The 600 mg SC pertuzumab dose determined in HMVs was confirmed in Part 2 of the study in patients with EBC.

Conclusions:
These data support the development of an SC pertuzumab + SC trastuzumab FDC product.
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Publication Number: P5-20-08

Title: HER2 in-Situ hybridization positive breast cancers with HER2/CEP17 ratio ≥2.0 and average HER2 copy number <4.0 are frequently discordant with HER2 immunohistochemistry results: Implications for potential modification of testing algorithm

Chad Livasy\(^1,2\), Nicole Johnson\(^2\) and Akosua Domfeh\(^2\). \(^1\)University of North Carolina, Chapel Hill, NC and \(^2\)Levine Cancer Institute, Charlotte, NC.

Body: Background: The current ASCO/CAP guidelines for the determination of HER2 status indicate that dual-probe in-situ hybridization (ISH) samples demonstrating a HER2/CEP17 ratio ≥ 2.0 and average HER2 copy number <4.0 must be reported as positive. Patients with tumors showing these rare features were included in the first generation adjuvant trastuzumab clinical trials. In the absence of data showing lack of efficacy of HER2-targeted in this small subset of patients, these cases continue to be classified as positive without requiring additional work-up. Given that the HER2/CEP17 ratio is skewed in these cases by complex segmental deletions in chromosome 17, additional work-up may improve the final HER2 classification of these tumors. The objective of this study is to assess the potential impact of integrating HER2 IHC into the testing algorithm for these tumors by correlating the HER2 IHC results with ISH positive breast cancers demonstrating HER2/CEP17 ratio ≥2.0 and average HER2 copy number <4.0.

Methods: All invasive mammary carcinomas demonstrating a HER2/CEP17 ratio ≥2.0 and average HER2 copy number <4.0 were identified from our database for years 2009-2017. All ISH testing was performed using the PathVysion HER2 DNA probe kit (Abbott Molecular). During the study interval, HER2 FISH was the institutional primary testing methodology to determine HER2 status. HER2 IHC (Ventana 4B5) was performed on the same sample for all identified cases and scored using 2013 ASCO/CAP scoring guidelines.

Results: A total of 96 (1.4%) of 6,976 consecutive FISH cases met criteria for inclusion into the study. The majority of cases tested negative (67%) or equivocal (29%) by IHC. HER2 protein overexpression was observed in 4% of cases. The results of HER2 IHC testing are summarized below.

<table>
<thead>
<tr>
<th>Cases (n)</th>
<th>IHC Score 0</th>
<th>IHC score 1+</th>
<th>IHC score 2+</th>
<th>IHC score 3+</th>
</tr>
</thead>
<tbody>
<tr>
<td>96</td>
<td></td>
<td>12 (13%)</td>
<td>52 (54%)</td>
<td>28 (29%)</td>
</tr>
</tbody>
</table>

Conclusions: HER2 ISH positive breast cancers demonstrating a HER2/CEP17 ratio ≥2.0 and average HER2 copy number <4.0 are rare (1.4% of cases in this study). HER2 IHC results for these tumors are negative in majority of cases. The observed HER2 protein overexpression rate was only 4% in this study. Integration of HER2 IHC into the testing algorithm for tumors demonstrating these uncommon ISH findings would result in reclassification of HER2 status to negative for most tumors.
Body: Background
A new subcutaneous (s.c.) formulation of trastuzumab became available in 2013 based on equivalent efficacy, pharmacokinetic (PK) profile and safety with the standard intravenous (i.v.) administration, where the s.c. trastuzumab was administered only into the thigh. As an s.c. injection into the abdominal wall (abdw) might be more convenient for patients (pts) and health care professionals, the PK profile of s.c. trastuzumab injected into the thigh vs the abdw in pts with HER2+ early BC needs to be evaluated.

Methods
GAIN-2 study compared two intense dose-dense (idd) anthracycline/taxane containing regimens. After completion of the anthracycline and i.v. trastuzumab given concurrently with taxanes, HER2+ BC pts were randomized in a 1:1 ratio to continue adjuvant s.c. trastuzumab 600mg fixed dose injected every 3 weeks either into the thigh or the abdw. Randomization was stratified according to CT arm [(iddEnPC) vs tailored dd CT (dtEC-dtD)] and age (≤ 50 vs >50). Pts in the EnPC arm received 14 and in the dtEC-dtD arm 15 cycles of s.c. trastuzumab.

For the PK profile of s.c. trastuzumab serum samples collected before cycle 7, on days 2, 4, 8, 15 and 21 of cycle 7 are evaluated. With a total sample size of 30 (15 per group), the simulated 90% two-sided CI for the area under the plasma concentration (AUC_0-last) will be (0.79-1.27) and for the peak drug concentration (C_{max}) will be (0.77-1.30). Allowing for a dropout rate of 15%, 18 pts per group will be included in the PK analysis.

The primary objective was to assess the PK profile of s.c. trastuzumab injected into the thigh vs the abdw. The secondary objectives included safety and tolerability.

Results
The per-protocol (pp) set consists of 30 pts (17 in the thigh group and 13 in the abdw group). Baseline characteristics were well balanced between the groups. The log-transformed Geometric Least Square Means (GLSM) for C_{max} were 11.77 and 11.52 in the thigh and the abdw group, respectively. The geo-mean ratio (on the original scale) for C_{max} was 1.29 (90% CI 1.05-1.58). The log-transformed GLSM for AUC_{0-last} were 14.54 and 14.28 in the thigh and the abdw group, respectively. The geo-mean ratio for AUC_{0-last} was 1.29 (90% CI 1.02-1.63).

Overall 29 pts (96.7%) reported any grade and 5 pts (16.7%) high grade adverse events (AEs). The incidence of any grade AEs was similar between the two groups. The most common AEs were anemia (70.6% for the thigh vs 61.5% for the abdw group, p=0.705), leukopenia (80.0% for both groups, p=1.000) and fatigue (47.1% for the thigh vs 76.9% for the abdw group, p=0.141). 6 serious AEs were reported (2 in the thigh vs 4 in the abdw group). The final PK results of s.c. trastuzumab will be presented at the meeting.

Conclusions
Bioavailability of s.c. trastuzumab as reflected by peak and total exposure measured in cycle 7 was approx. 30% higher if administered into the thigh than into the abdw in pts with HER2+ primary BC treated after dose-dense CT plus i.v. trastuzumab. However, no increased toxicity was observed. Study limitations were that no cross-over design was used and number of pts satisfying criteria for pp-set were different in the arms.
Patterns of use and effectiveness of neoadjuvant pertuzumab-containing regimens in stage II-III HER2-neu positive breast cancer: A retrospective, single institutional experience

Murthy K Rashmi1, Raghavendra S Akshara1, Hess R Kenneth1, Hong Z Amy1, Lim Bora1, Barcenas H Carlos1, Chavez-MacGregor Mariana1, Mittendorf A Elizabeth1, Litton K Jennifer1, Giordano H Sharon1, Thompson Alastair1, Valero Vicente1, Tripathy Debu1 and Ueno T Naoto1. 1The University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Background: Pertuzumab (P) in combination with trastuzumab (H) based chemotherapy is currently FDA-approved as a standard neoadjuvant treatment for patients with clinical stage II-III HER2-positive (HER2+) breast cancer (BC). The chemotherapy backbone of HER2-targeted therapy varies and includes a taxane (T) and/or an anthracycline coupled with cyclophosphamide (A), or carboplatin (C). The goal of this study was to retrospectively evaluate patterns of use and the pathologic complete response (pCR) rate for HP-containing regimens comparing the 3 treatment groups: A-containing, C-containing, and taxane only for stage II-III HER2+ BC.

Methods: We identified all patients (n=226) with stage II-III non-inflammatory HER2+ BC who received neoadjuvant HER2-targeted therapy in combination with P and H from 2013 to 2016 through an institutional database. 48 received THP, 85 received TCHP, 5 received TCHP-A, and 88 received THP-A. All patients underwent definitive breast and lymph node surgery. Medical records were examined for patient demographics, breast cancer stage, pathology results, surgical outcomes, and treatment details. pCR was defined as ypT0/is, ypN0. Descriptive statistics and logistic regression analysis was used for statistical analysis.

Results: Patient characteristics are shown in Table 1. The median age was 51 (22-84) years. The pCR rates by regimen were as follows: THP (65%), TCHP (50%), THP-A (58%). After controlling for age, stage, HR status, grade, and menopausal status, THP was associated with a modestly higher pCR rate compared to TCHP or THP-A: OR = 1.82 (0.88, 3.74)(p = 0.11). Efficacy and toxicity differences will continue to be evaluated as more analytic cases become available at our institution.

Conclusion: Although it is challenging to demonstrate non-inferiority without a prospective randomized trial, THP, a regimen not containing carboplatin or anthracycline, appears to have similar efficacy in selected patients with stage II-III HER2+ BC. Further research is needed to identify biomarkers to select patients appropriate for de-escalation of therapy.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Anthraclyline Containing (THP-A) N=88</th>
<th>Carboplatin Containing (TCHP; TCHP-A*) N=90</th>
<th>Taxane alone (THP) N=48</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at Diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 50</td>
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<td>60%</td>
</tr>
<tr>
<td>&gt; 50</td>
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<td>Clinical State at Diagnosis</td>
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<tr>
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<td>34%</td>
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<tr>
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<td>23%</td>
</tr>
<tr>
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<tr>
<td>IIC</td>
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<td>18%</td>
<td>19%</td>
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<td>-----</td>
</tr>
<tr>
<td></td>
<td>III</td>
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<td>66%</td>
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<tr>
<td></td>
<td>HR(-)</td>
<td>42%</td>
<td>33%</td>
</tr>
</tbody>
</table>

*5/90 received TCHP-A; **3 patients did not have nuclear grade available; 3 patients had nuclear grade 1 cancer
Title: Comparative effectiveness of neoadjuvant therapy for HER2-Positive breast cancer: Addition of new clinical evidence to network meta-analysis and data update after 5 years

Ayako Nakashoji¹, Tetsu Hayashida¹, Takamichi Yokoe¹, Hinako Maeda¹, Rurina Watanuki¹, Masayuki Kikuchi¹, Tomoko Seki¹, Maiko Takahashi¹, Takayuki Abe² and Yuko Kitagawa¹. ¹Keio University School of Medicine and ²Keio University School of Medicine.

Body: Background: It is becoming more popular to perform neoadjuvant chemotherapy including anti-HER2 agents to operable HER2-positive breast cancer patients. Increasing HER2-targeted treatment options urge us to define the best neoadjuvant therapy. In 2014, we reported the systematical assessment of the efficacy and safety of neoadjuvant therapy for HER2-positive breast cancer, using network meta-analysis based on Bayesian model (Nagayama et al., JNCI 2014). Network meta-analysis synthesizes information from a network of trials, which helps interpret the randomized evidence and can rank treatments from different trials. After five years from our first literature search, we decided to update our analysis due to accumulation of new clinical evidence.

Methods: We assessed odds ratio for pathological complete response (pCR), completion, and safety in seven treatment arms utilizing pooling effect sizes. The treatment arms included the combinations of chemotherapy (CT), trastzumab (tzmb), lapatinib (lpnb) and pertzumab (pzmb). All statistical tests were two-sided, and we followed Preferred Reporting Items for Systematic Reviews and MetaAnalyses (PRISMA) guidelines.

Results: A database search identified 993 articles with 13 studies meeting the eligibility criteria, adding three studies (a trial of CT + tzmb vs CT + lpnb, and two trials of CT + tzmb vs CT + lpnb vs CT + tzmb + lpnb) to previous analysis. In direct comparison, CT + tzmb significantly achieved more pCR than CT + lpnb (OR=0.68, 95% CI = 0.52 to 0.89, p=.005) despite no statistical difference was found previously. In indirect comparison, treatment arms of dual anti-HER2 agents with CT achieved more pCR than other arms, reducing their credibility intervals against all other arms. This trend was stronger in CT + tzmb + lpnb arm (CT + tzmb + lpnb vs CT + tzmb, OR = 1.62, 95% CrI = 1.19 to 2.22, p = .003), which we added sufficient clinical evidence. Moreover, it exposed the need for additional clinical data for pzmb relative arms. Values of surface under the cumulative ranking (SUCRA) suggested that CT + tzmb + pzmb had the highest probability of being the best treatment arm for pCR (SUCRA = 0.95), followed by CT + tzmb + lpnb (SUCRA = 0.87), and CT + tzmb (SUCRA = 0.62), widening the gap and differentiating the top two dual blockade arms which were close in our previous report. All outcomes from our present analysis were consistent with our previous report and strengthened data solidity by reducing confidence or credibility intervals.

Conclusion: Consistent results in not only in pCR but also in completion rates and adverse events indicate that we are looking at the results which are close to the truth. Additional trials of lpnb relative regimens are not probable to change the results, but pzmb relative trials are required to improve evidence solidity. New clinical data established stronger evidence in network meta-analysis that combining two anti-HER2 agents with CT is most effective in the neoadjuvant setting for HER2-positive breast cancer.
**Title:** Adjuvant DCH vs TCH for low-risk (node negative); and FECDH vs TCH for high-risk (node positive) HER2+ breast cancer – A retrospective provincial analysis

Zachary W Veitch¹, Omar F Khan¹, Derek Tilley², Xanthoula Kostaras², Patricia A Tang¹, Karen King³ and Sasha Lupichuk¹. ¹Tom Baker Cancer Centre - University of Calgary, Calgary, AB, Canada; ²Alberta Health Services - Cancer Control, Edmonton, AB, Canada and ³Cross Cancer Institute - University of Alberta, Edmonton, AB, Canada.

**Body:**

**Background:** Chemotherapy plus trastuzumab for early HER2+ breast cancer (BC) is associated with improved survival. Optimal regimens for low-risk (node negative) and high-risk (node positive) HER2+ breast cancers are unknown and choice of regimen varies in real-world clinical practice.

**Objective:** (1) For low-risk breast cancer, to compare DCH (4 cycles) and TCH (6 cycles) in terms of disease free (DFS) and overall survival (OS). (2) For high-risk breast cancer, to compare FECDH (6 cycles) and TCH (6 cycles) in terms of DFS and OS.

**Methods:** All women diagnosed from 2007-2014 with stage I-III, hormone receptor (HR) +/-, HER2+ BC receiving adjuvant chemotherapy plus trastuzumab (n=986) in Alberta, Canada were included. Patients with low-risk (node negative) disease were stratified into DCH (n=104) or TCH (n=360) cohorts for DFS/OS comparison (Kaplan-Meier). Patients with high-risk (node positive) disease were stratified into FECDH (n=145) or TCH (n=314) cohorts. Subgroup analysis of the high-risk cohorts by HR+/HER2+ and HR-/HER2+ for FECDH vs TCH were performed. Chi-square was used to evaluate for difference between cohort variables.

### Low-Risk Cohort

<table>
<thead>
<tr>
<th></th>
<th>DCH</th>
<th>TCH</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (104)</td>
<td>%</td>
<td>n (360)</td>
</tr>
<tr>
<td>Age (mean)</td>
<td>55.3</td>
<td>53.0</td>
</tr>
<tr>
<td>Hormone Status</td>
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</tr>
<tr>
<td>ER+ or PR+</td>
<td>86</td>
<td>276</td>
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<tr>
<td>ER and PR-</td>
<td>18</td>
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<tr>
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<td>mastectomy</td>
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<td>246</td>
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### High-Risk Cohort

<table>
<thead>
<tr>
<th></th>
<th>FECDH</th>
<th>TCH</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (145)</td>
<td>%</td>
<td>n (314)</td>
</tr>
<tr>
<td>Age (mean)</td>
<td>50.2</td>
<td>53.6</td>
</tr>
<tr>
<td>Hormone Status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER+ or PR+</td>
<td>115</td>
<td>79.3</td>
</tr>
<tr>
<td>------------</td>
<td>-----</td>
<td>------</td>
</tr>
<tr>
<td>ER and PR-</td>
<td>30</td>
<td>20.7</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Grade</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
<td>29</td>
<td>116</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>20</td>
<td>80</td>
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<tr>
<td></td>
<td>5</td>
<td>61</td>
<td>146</td>
</tr>
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</table>

<table>
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<tr>
<th>Surgery</th>
<th>lumpectomy</th>
<th>39</th>
<th>27.1</th>
<th>98</th>
<th>31.2</th>
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<tbody>
<tr>
<td></td>
<td>mastectomy</td>
<td>105</td>
<td>72.9</td>
<td>216</td>
<td>68.8</td>
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<table>
<thead>
<tr>
<th>Node Status</th>
<th>N1</th>
<th>N2</th>
<th>N3</th>
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<tbody>
<tr>
<td></td>
<td>83</td>
<td>41</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>57.2</td>
<td>28.3</td>
<td>14.5</td>
</tr>
<tr>
<td></td>
<td>194</td>
<td>73</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>61.8</td>
<td>23.2</td>
<td>15</td>
</tr>
</tbody>
</table>

**Results:** Median follow-up was 58.1 months in the low-risk cohort and 63.1 months in the high-risk cohort. In the low-risk group, patients receiving TCH had more mastectomy (69.5%) than lumpectomy (30.5%; p<0.001) compared to those receiving DCH (50%; 50%). No significant difference was seen in DFS (p=0.153) or OS (p=0.409) for patients in the DCH (92.3%; 95.2%) vs TCH (95.2%; 96.9%) cohorts. In the high-risk group, no significant difference was seen in DFS (p=0.226) or OS (p=0.164) for FECDH (92.4; 95.2%) or TCH (88.5%; 91.4%) respectively. In subgroup analysis of high-risk HR+/HER2+ BC, patients receiving FECDH demonstrated superior OS (98.3%; p=0.014) and a trend towards superior DFS (94.8%; p=0.069) relative to TCH patients (OS = 91.6%; DFS= 88.7%). Conversely, analysis of high-risk HR-/HER2+ BC, patients demonstrated higher DFS and OS for TCH (88.2%; 90.8%) relative to FECDH (83.3%; 83.3%); although this was non-significant (p=0.516; p=0.298) and likely underpowered. Nodal status was balanced between all groups (p=0.602).

**Conclusion:** In low-risk HER2+ BC, 4 cycles of DCH chemotherapy has high survival with similar outcomes to 6 cycles of TCH. In high-risk HER2+ BC, FECDH has comparable outcomes to TCH consistent with BCIRG-006. This study suggests that women with HR+/HER2+ breast cancer have improved OS with anthracycline containing regimens, such as FECDH. Although non-significant, patients with HR-/HER2+ BC may have some improvement in DFS and OS with TCH, a carboplatin containing regimen.
2017 San Antonio Breast Cancer Symposium

Publication Number: P5-20-13

Title: Biosimilar ABP 980 in patients with early breast cancer: Results of single switch from trastuzumab to ABP 980

Gunter von Minckwitz¹, Maria Turdean², Nan Zhang³, Patricia Santi⁴ and Vladimir Hanes³. ¹German Breast Group, Neu-Isenburg, Germany; ²Emergency County Hospital Cluj, Cluj-Napoca, Romania; ³Amgen, Inc., Thousand Oaks, CA and ⁴Centro de Estudios de Hematologiae Oncologia, Sao Paulo, Brazil.

Body: Background: Analytical, functional, and pharmacokinetic similarity between ABP 980 and trastuzumab (TRAS) has been demonstrated. Here we report results from the single switch treatment arm in the adjuvant phase of the corresponding clinical study.

Methods: The objective of this randomized, multicenter, double-blind study was to compare ABP 980 with TRAS in women with HER2-positive early breast cancer. After run-in anthracycline-based chemotherapy, patients were randomized 1:1 to intravenous ABP 980 or TRAS plus paclitaxel Q3W for 4 cycles. Surgery (breast and sentinel node or axillary lymph node dissection) was completed 3-7 weeks after the last dose of study drug. Following surgery, patients who initially received TRAS were allocated to either continue TRAS or undergo a single switch to receive ABP 980 Q3W for up to 1 year from the first dose of investigational product (IP) in the neoadjuvant phase. Allocation occurred at randomization and was maintained blinded. The objective of the single switch was to evaluate safety and immunogenicity of subjects transitioning from TRAS to ABP 980.

Results: Here we report data collected at the time of the primary analysis, when all patients had completed the first post-surgery clinical visit or withdrawn from the study. The majority of patients had completed the study at the time of this analysis. Of the 827 enrolled patients, 725 were randomized (ABP 980: n=364; TRAS: n=361). Of the 361 patients randomized to TRAS in the neoadjuvant phase, 173 and 174 patients in the TRAS/TRAS and TRAS/980 arm completed surgery, respectively, and 171 patients in each group entered the adjuvant phase following surgery. A total of 89 (52.0%) and 98 (57.3%) patients had an adverse event (AE) in the TRAS/TRAS and TRAS/980 group, respectively; 10 (5.8%) patients in each group had a grade ≥3 AE.

AEs of interest are listed in Table 1.

Table 1: Adverse Events of Interest

<table>
<thead>
<tr>
<th></th>
<th>TRAS/980 (n = 171)</th>
<th>TRAS/TRAS (n = 171)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total, n (%)</td>
<td>Grade ≥3, n (%)</td>
</tr>
<tr>
<td>Any AE of interest</td>
<td>45 (26.3)</td>
<td>5 (2.9)</td>
</tr>
<tr>
<td>Infections and Infestations</td>
<td>21 (12.3)</td>
<td>2 (1.2)</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>13 (7.6)</td>
<td>1 (0.6)</td>
</tr>
<tr>
<td>Infusion reactions</td>
<td>15 (8.8)</td>
<td>3 (1.7)</td>
</tr>
<tr>
<td>Hypersensitivity</td>
<td>7 (4.1)</td>
<td>0</td>
</tr>
<tr>
<td>Pulmonary toxicity</td>
<td>1 (0.6)</td>
<td>1 (0.6)</td>
</tr>
<tr>
<td>Cardiac failure</td>
<td>1 (0.6)</td>
<td>0</td>
</tr>
</tbody>
</table>

One patient in the TRAS/980 arm developed binding, non-neutralizing anti-drug antibodies (ADAs) during the adjuvant phase, compared with 4 patients (2 each in the ABP 980 and TRAS arms) in the neoadjuvant phase. The percentage of patients with disease progression or recurrence or death was 5.3% and 2.9% in the TRAS/TRAS and TRAS/980 arm, respectively (hazard ratio for TRAS/980 vs TRAS/TRAS, 0.48; 90% CI: [0.181, 1.292]).

Conclusions: Switching from TRAS to ABP 980 following surgery was safe in patients with breast cancer. Switching did not increase the frequency or severity of AEs and no unexpected safety signals were noted, and it did not increase the incidence of developing ADAs. Event-free survival was also similar between treatment groups.
Title: Cardiotoxicity in 1 year of treatment with reference trastuzumab and its biosimilar candidate CT-P6 in HER2 positive early stage breast cancer (EBC) patients

Body: Background CT-P6 is a proposed biosimilar to reference trastuzumab. This trial (NCT02162667) evaluated the similarity of CT-P6 and reference trastuzumab for both efficacy and safety in HER2 positive EBC patients. The primary endpoint, pathological complete response (pCR) rate was entirely within the pre-defined equivalence margin (Stebbing et al., Lancet Oncology 2017). Efficacy and safety were similar between the two treatment groups. We aimed to investigate the cardiotoxicity in the 1 year treatment and follow-up period.

Methods A total of 549 patients with HER2 positive EBC were randomized to receive CT-P6 (n=271) or reference trastuzumab (n=278) in combination with docetaxel (Cycles 1-4) and 5-fluorouracil, epirubicin and cyclophosphamide (FEC, Cycles 5-8) in the neoadjuvant setting. CT-P6 or reference trastuzumab was administered at 8 mg/kg (Cycle 1 only) followed by 6 mg/kg every 3 weeks. After surgery, patients received monotherapy of CT-P6 or reference trastuzumab up to 10 cycles in the adjuvant setting. Left ventricular ejection fraction (LVEF) was evaluated by ECHO or MUGA at baseline, every 3 or 4 cycles during treatment and every 6 months thereafter. If LVEF decreased by ≥10 points from baseline and <50%, a reassessment was performed within 3 weeks and the patient was withdrawn from the study treatment if the cardiac toxicity was confirmed.

Results Median follow-up period was 19 months. In total, 96% of patients completed 8 cycles of taxane and anthracycline combination therapy during the neoadjuvant period and 90% of patients in each group received 1-year treatment of CT-P6 or reference trastuzumab. The relative dose intensity of study drug was similar, indicating good tolerability of CT-P6 and reference trastuzumab (97.5% and 97.3% during the neoadjuvant period; 98.5% and 98.8% during the adjuvant period). Docetaxel and FEC combination therapies were administered approximately 96% of dose intensity in the two groups. All patients had normal LVEF (≥55%) at baseline. Mean LVEF value was maintained more than 60% during 1-year treatment and follow-up period. Heart failure cases with LVEF decrease (≥10 points from baseline and <50%) were reported to be similar between two groups. Three patients in each group were withdrawn due to LVEF decrease. Adverse events of cardiac disorders were reported to be similar between two groups. All cases were mild or moderate except two cases.

Table 1. Summary of heart failure with LVEF decrease and cardiotoxicity

<table>
<thead>
<tr>
<th>LVEF decrease ≥ 10 points and below 50%</th>
<th>CT-P6 (N=271)</th>
<th>Reference Trastuzumab (N=278)</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Asymptomatic</td>
<td>9 (3.3%)</td>
<td>6 (2.1%)</td>
</tr>
<tr>
<td>- Symptomatic</td>
<td>0</td>
<td>1 (0.4%)</td>
</tr>
<tr>
<td>- Confirmed</td>
<td>2 (0.7%)</td>
<td>4 (1.4%)</td>
</tr>
<tr>
<td>- Withdrawn</td>
<td>3 (1.1%)</td>
<td>3 (1.1%)</td>
</tr>
<tr>
<td>Cardiac disorder by AEs</td>
<td>31 (11.4%)</td>
<td>38 (13.7%)</td>
</tr>
<tr>
<td>- Grade 1 to 2</td>
<td>30 (11.0%)</td>
<td>37 (13.3%)</td>
</tr>
<tr>
<td>- Grade 3 to 5</td>
<td>1 (0.4%)</td>
<td>1 (0.4%)</td>
</tr>
</tbody>
</table>

1 Grade 3 of Adams-Strokes syndrome occurring 5 months after the completion of 1-year treatment; 2 Grade 5 of acute myocardial infarction occurring 10 days after Cycle 1 of the neoadjuvant therapy

Conclusions Combination therapy of CT-P6 with taxane/anthracycline and monotherapy of CT-P6 over 1 year period were well tolerated and cardiotoxicity was similar to reference trastuzumab.
Title: Dosing patterns and economic burden of drug wastage among postmenopausal women with HR+/HER2- metastatic breast cancer receiving palbociclib

Anand A Dalal¹, Patrick Gagnon-Sanschagrin², Rebecca Burne², Annie Guerin², Genevieve Gauthier², Tania Small¹ and Polly Niravath³. ¹Novartis Pharmaceuticals Corporation, East Hanover, NJ; ²Analysis Group, Montreal, QC, Canada and ³Houston Methodist Hospital, Houston, TX.

Body: Background: Dose modification related to adverse event is common in the treatment of metastatic breast cancer (mBC). Based on the dosage form and strengths available, dose modification may lead to drug wastage when the dose cannot be split or saved for later use. This study aimed to describe dosing patterns and to estimate the economic burden of drug wastage associated with dose modifications in postmenopausal women with HR+/HER2- mBC receiving palbociclib.

Methods: Postmenopausal adult women diagnosed with HR+/HER2- mBC were identified from the Truven administrative claims database (2006Q1–2015Q4). Regimens received following mBC diagnosis were identified – patients who received a palbociclib-based regimen during one of their first three lines of therapy for mBC were included in the study. Palbociclib starting daily dose, average daily dose, and dosing patterns (dosing modifications and sequences) were reported. A dose modification was defined as a change (decrease/increase) of ≥25mg daily compared to the preceding dose. The economic burden of drug wastage was estimated by multiplying the number of days with drug wastage (i.e., days with overlapping palbociclib prescriptions due to dosage change) by the average cost reimbursed by payers for one unit of palbociclib. Descriptive analyses were conducted separately by line of therapy for mBC.

Results: A total of 473 patients received palbociclib in first (214), second (157), or third (120) line of therapy for mBC. Patients were observed to receive palbociclib for an average of 4.3 months in first line and 4.1 months in second and third line of therapy. The majority of patients started palbociclib on the recommended 125 mg dose and remained on that dose until the end of observation. Dosing patterns and sequences are summarized in Table 1. Among the 214 patients who used palbociclib in first line, 38 (17.8%) had a dose modification – among these, 6 (15.8%) patients had an overlap in prescription fills (average overlap of 9.2 days). This potential drug wastage resulted in an average cost of $4,376 per patient over a period of approximately 4 months following treatment initiation. Results were consistent in second and third lines of therapy, with higher proportions of patients with drug wastage in later lines of therapy.

Conclusion: Over a short observation period, dose modifications, mostly dose reductions, were relatively frequent, and potential resulting drug wastage was associated with a substantial economic burden.

Table 1: Palbociclib dosing patterns and sequences

<table>
<thead>
<tr>
<th>Starting dose (mg daily), N (%)</th>
<th>First line</th>
<th>Second line</th>
<th>Third line</th>
</tr>
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<tbody>
<tr>
<td>75</td>
<td>7 (3.3)</td>
<td>3 (1.9)</td>
<td>3 (2.5)</td>
</tr>
<tr>
<td>100</td>
<td>15 (7.0)</td>
<td>17 (10.8)</td>
<td>4 (3.3)</td>
</tr>
<tr>
<td>125 - recommended dose</td>
<td>192 (89.7)</td>
<td>137 (87.3)</td>
<td>113 (94.2)</td>
</tr>
<tr>
<td>Average dose on treatment, mean±SD [median]</td>
<td>119.52±11.27 [125]</td>
<td>115.95±13.61 [125]</td>
<td>116.85±13.30 [125]</td>
</tr>
<tr>
<td>Dose sequencing (mg daily), N (%)</td>
<td>163 (76.2)</td>
<td>96 (61.1)</td>
<td>74 (61.7)</td>
</tr>
<tr>
<td>125→100</td>
<td>21 (9.8)</td>
<td>29 (18.5)</td>
<td>26 (21.7)</td>
</tr>
<tr>
<td>125→100→75</td>
<td>6 (2.8)</td>
<td>8 (5.1)</td>
<td>7 (5.8)</td>
</tr>
<tr>
<td>125→100→125</td>
<td>0 (0.0)</td>
<td>1 (0.6)</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>125→75</td>
<td>2 (0.9)</td>
<td>3 (1.9)</td>
<td>4 (3.3)</td>
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<tr>
<td>Path</td>
<td>Visits</td>
<td>P&gt;</td>
<td>V</td>
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<tr>
<td>125→75→100</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
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2017 San Antonio Breast Cancer Symposium

Publication Number: P5-20-16

Title: Improving the delivery of trastuzumab to breast cancer tumors with a bottom-up, multi-scale systems pharmacological analysis of sequence combinations

Sihem Ait-Oudhia. 1 College of Pharmacy. University of Florida, Orlando, FL.

Body: Background: One of the major continuous challenges in combination chemotherapy is the inadequate delivery and target site penetration of anticancer drugs to solid tumors. To date, the dose-, time-, and sequence-dependence of combination therapies have been established empirically. This presentation describes a successful example of the use of a bench-to-bed side, systems pharmacological approach for optimization of sequence treatment of a tumor priming agent (Paclitaxel, PAC) to enhance the penetration and efficacy of a subsequent monoclonal antibody (Trastuzumab, TmAb).

Method: Six therapeutic regimens were investigated in vitro on BT474, a breast cancer cell line overexpressing HER2 receptors, and in vivo on Balbc nude mice xenografted with the same, including: 1) tumor priming regimen (TPR) with PAC administered 24 h prior to TmAb, 2) reverse-TPR with TmAb administration first then PAC 24 h later, 3) simultaneous administration of both drugs, 4) PAC alone, 5) TmAb alone, 6) vehicle as a control. The anti-tumoral responses in all treatment arms were monitored over time. They included the measurement of: 1) selected key intracellular signaling proteins in the HER2 pathway, 2) cellular responses such as apoptosis, cell cycle arrest, and antibody dependent cellular cytotoxicity (ADCC), 3) the reduction in tumor volumes, and 5) animals survival. All multi-level data were integrated into a comprehensive multi-scale quantitative systems pharmacology (QSP) model. The later served as a simulation tool for identification in silico of an optimized inter-dose interval for tumor priming with PAC and sequence combination with TmAb.

Results: In vitro and in vivo results showed that TPR arm was superior to all other regimens. The proteins P21, a hallmark biomarker of cell-cycle arrest, and cleaved-PARP, a late apoptotic biomarker, were constitutively activated in the TPR arm. Furthermore, apoptosis and ADCC responses were enhanced in the same. In vivo studies showed a greater tumor volume shrinkage response in the animals from the TPR treatment arm compared to the other therapeutic schemes and a longer animal survival. Mathematical modeling results identified a synergistic drug-drug interaction between PAC and TmAb while given in a TPR with 24 h interval.

Conclusion: The superior anti-tumor responses from the TPR compared to the other regimens are promising. The final QSP analysis recapitulated with remarkable fidelity the greater efficacy of TPR compared to control groups in vivo. Clinical investigations are needed to determine its applicability to HER2+ Breast cancer patient population.
Body: Introduction: Trastuzumab is a monoclonal antibody that targets the human epidermal growth factor receptor (HER)-2 and significantly improves survival in HER-2 positive early stage breast cancer. However, trastuzumab is also known to cause cardiotoxicity. Guidelines recommend withholding or discontinuing trastuzumab if left ventricular ejection fraction (LVEF) falls ≥15% from baseline, or to <50%, but there is little evidence to support this strategy. Additionally, premature discontinuation of trastuzumab may lead to poorer cancer outcomes. Trastuzumab cardiotoxicity is frequently reversible, and the use of angiotensin converting enzyme-inhibitors (ACE-I) and beta blockers is highly effective at treating impaired LVEF in other patient populations. We therefore hypothesize that it is safe to continue trastuzumab in patients with asymptomatic or mildly symptomatic fall in LVEF, when concomitantly treated with ACE-I and beta blockers.

Methods: In this retrospective chart review, we identified 18 consecutive patients with stage 1-3 HER-2 positive breast cancer patients who had a decline in LVEF meeting the criteria above to withhold trastuzumab, and who were referred to our cardio-oncology service from the beginning of 2015 to March 2017. These patients were offered and consented to receive ongoing trastuzumab accompanied by ACE-I and/or beta blocker. Data on patient demographics, cancer therapies, clinical features, LVEF, and cardiac medications were extracted from medical charts.

Results: Among the 18 patients identified, all were women, 12 (67%) were estrogen receptor positive, and 7 progesterone receptor positive (39%). 11 (61%) of patients had a left-sided breast cancer, 6 (33%) had right-sided breast cancer, and 1 (5%) had an unspecified side breast cancer. 17 (94%) of patients had previously undergone radiation therapy, and 4 (22%) had experienced a recurrence. 16 patients had received a prior anthracycline regimen (10 sequentially, 6 concurrently). The patients were treated with one or a combination of the following medications: carvedilol (n=13), ramipril (n=12), bisoprolol (n=1), candesartan (n=2), rosuvastatin (n=2), atorvastatin (n=2). The patients were followed up for an average of 7 months after starting cardiac therapy. Table 1 summarizes the mean±SD LVEF values over the follow up time period. All patient except one (94%) completed trastuzumab treatment successfully. This patient with concomitant moderate-severe mitral regurgitation was hospitalized for pulmonary edema. Trastuzumab was discontinued and LVEF has subsequently returned to normal values.

Table 1: LVEF progression from baseline to end of follow-up

<table>
<thead>
<tr>
<th></th>
<th>Mean±SD LVEF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD LVEF at baseline</td>
<td>57.59±5.49%</td>
</tr>
<tr>
<td>Mean±SD LVEF on referral to cardio-oncology clinic</td>
<td>50.75±4.76%</td>
</tr>
<tr>
<td>Mean±SD LVEF at end of follow-up</td>
<td>56.35±3.50%</td>
</tr>
</tbody>
</table>

Conclusion: Treating cardiac dysfunction related to trastuzumab in a cardio-oncology clinic, using beta blockers and ACE-I may enable completion of trastuzumab therapy. Randomized trials are necessary before it can be widely recommended, however findings from our experience suggest this may be a promising new treatment strategy.
Title: Comparison of paclitaxel plus T-DM1 and pertuzumab versus paclitaxel plus trastuzumab and pertuzumab with carboplatin for HER2 overexpressed breast cancer models

Yuliang Sun¹, Xiaqian Lin¹, Tyler Jepperson¹, Casey Williams¹, Pradip De¹ and Brian Leyland-Jones¹. ¹Avera Cancer Institute, Sioux Falls, SD.

Body: Background: Remarkable progressions have been made in the breast cancer treatments over the past years, however, patients getting relief from the toxicity of cancer treatments are still unmet needs. The neoadjuvant regimen of docetaxel, carboplatin, and trastuzumab plus pertuzumab is standard choice for patients with HER2 positive breast cancer (Phase III results of the KRISTINE trial, SA Hurvitz et al., 2016). Trastuzumab emtansine (T-DM1) plus pertuzumab (P) has shown superior anti-tumor effect comparing with trastuzumab/Herceptin (H) plus P in preclinical HER2 positive breast cancer models (Y Sun et al., 2013). In this study we aimed to evaluate the preclinical efficacy of paclitaxel (T) plus T-DM1 and P versus paclitaxel plus H and P with carboplatin (C) (TCHP) in HER2 positive breast cancer models. Methodology: 1) HER2 amplified/overexpressed with ER+ BT474 (H-sensitive) and BT474HerR, an H-resistant derivative from BT474, were used. 2) 6-7 week-old female NCr (nu/nu, 20-25g) athymic mice (Taconic) were used for in vivo tumor growth inhibition studies. 3) Mice were injected s.c. on the flank with 5 x 10⁶ BT474 or BT474HerR cells. All mice were also received s.c. injection of 1 mg/kg estradiol valerate (JHP Pharmaceuticals) once weekly to promote tumor cell growth. 4) Mice bearing BT474 or BT474HerR tumors were treated with different combinations of T (20 mg/kg, i.v., every 4 days, total 7 doses), C (40 mg/kg, i.v., one dose), H (10 mg/kg, i.p., twice weekly for 4 weeks), T-DM1 (10 mg/kg, i.v., every 3 weeks, total 3 doses) and P (15 mg/kg, i.p., once weekly for 8 weeks) versus vehicle control. 5) In vivo data were analyzed by Student's t-test. A p-value of 0.05 was considered statistically significant. Results: We observed that 1) both regimens of TCHP and paclitaxel plus T-DM1 and P inhibited tumor growth in BT474 (TCHP p<0.0043 vs. T+T-DM1+P p<0.0025) and BT474HerR (TCHP p<0.00044 vs. T+T-DM1+P p<0.000037) xenograft models; 2) T+T-DM1+P caused almost complete regression in both models (comparing to 1 day before treatment started, the mean tumor volume at the end of study was decreased ~ 91.13% in BT474 model and ~ 100% in BT474HerR model) whereas TCHP caused significant tumor growth inhibition in both models (comparing to 1 day before treatment started, the mean tumor volume at the end of study was decreased ~ 74.6% in BT474 model and ~ 73.31% in BT474HerR model). Conclusion: Our in vivo data suggest that 1) T+T-DM1+P shows equivalent excellent antitumor activity to TCHP in HER2 overexpressed BT474 model; 2) T+T-DM1+P shows superior antitumor activity to TCHP (p<0.0045) in HER2 overexpressed BT474HerR model; 3) comparing to TCHP, the combination of T+T-DM1+P may have better safety profile in patients.
**Title:** PAM50 intrinsic subtype predicts survival outcome in HER2-positive/hormone receptor-positive metastatic breast cancer treated with palbociclib and trastuzumab: a correlative analysis of the PATRICIA (SOLTI 13-03) trial

**Body:**

**Background.** In HER2+/HR+ breast cancer (BC), CDK4/6 inhibition combined with anti-HER2 therapy is currently being explored in phase II/III trials. However, we and others have shown that Luminal A and B subtypes (i.e. luminal disease) defined by gene expression only represent 30-50% in this group. Identification of the luminal subtype in HER2+/HR+ disease might be important since the median IC50 of palbociclib in HER2+ BC cell lines falling into the luminal subtype is lower than in non-luminal HER2+ cell lines (47.5 vs. 300 nM; Finn BCR 2011). Here, we explored, for the first time, the efficacy (progression-free survival [PFS]) of palbociclib and trastuzumab and its association with subtype in HER2+/HR+ BC.

**Methods.** PATRICIA is an exploratory, prospective, open-label, multicenter phase II trial in advanced HER2+ BC. Patients (pts) had received 2-4 prior lines of anti-HER2-based regimens. Treatment consisted of trastuzumab 6 mg/kg every 3w and palbociclib 200 mg daily for 2w and 1w off. The study was based on a Simon 2-stage design comprising 3 cohorts: ER-negative cohort A (still recruiting) and ER+ cohorts B1 and B2 (both completed). Patients in cohort B2 also received letrozole by randomization. Safety run-in phase was made for first 12 pts. For stage 1 to be successful, at least 6 pts of 15 had to be progression-free at 6 months (PFS6) in each cohort. In stage 2, up to 46 pts per cohort will be included to show a PFS6 >50%. As a secondary objective, research-based PAM50 intrinsic subtype was performed from FFPE samples using the nCounter platform. Estimates of PFS were from Kaplan-Meier curves. Univariate Cox regression analyses evaluating luminal subtype, age, performance status, treatment line, type of biopsy and endocrine treatment were evaluated.

**Results.** Thirty-one pts with HER2+/HR+ disease were recruited in stage 1 in cohorts B1 (n=16) and B2 (n=15). Median age was 59.8y, and median number of prior lines was 3.0. The rate of PFS6 was 40.0% (6/15) and 53.3% (8/15) in cohorts B1 and B2, respectively. A total of 26 (83.9%) tumors samples (15 primary and 11 metastatic tumors) were profiled. Subtype distribution was as follows: 46.2% HER2-enriched, 23.1% Luminal B, 19.2% Luminal A and 11.5% Normal-like. Median PFS in this subgroup of patients with PAM50 data was 3.71 months. Compared to non-luminal disease, luminal disease showed a statistically significantly longer median PFS (10.37 vs. 3.53 months, p-value=0.023). The PFS hazard ratio of luminal versus non-luminal groups was 0.34 (95% CI 0.13-0.92, p-value=0.033). In addition, Luminal A signature score, as a continuous variable, was also found associated with PFS (adjusted hazard ratio=0.54, 0.31-0.92, p-value=0.023). No clinical-pathological variable was found associated with PFS.

**Conclusion.** PAM50 subtype predicts PFS in HER2+/HR+ advanced BC treated with palbociclib and trastuzumab. Patients with non-luminal disease might not derive a large benefit from this treatment strategy concordant with the preclinical in vitro data. Our results might have important implications for current and future clinical trials evaluating CDK4/6 inhibitors in HER2+/HR+ disease.
Title: Cobimetinib combined with paclitaxel as first-line treatment for patients with advanced triple-negative breast cancer (COLET study): Primary analysis of cohort I

Adam Brufsky¹, David Miles², Zanete Zvirbule³, Alexandru Eniu⁴, Elena Lopez-Miranda⁵, Jae Hong Seo⁶, Michele Orditura⁷, Fanny Le Du⁸, Matthew Wongchenko⁹, Melanie Poulin-Costello¹⁰, Brian Simmons¹, Virginia McNally¹¹, Sherene Loi¹² and Sung-Bae Kim¹³. ¹University of Pittsburgh Cancer Institute, Pittsburgh, PA; ²Mount Vernon Cancer Centre, London, United Kingdom; ³Riga East Clinical University Hospital Latvian Oncology Centre, Riga, Latvia; ⁴Cancer Institute “Ion Chiricuta”, Cluj-Napoca, Romania; ⁵Hospital Universitario Ramon y Cajal, Madrid, Spain; ⁶Korea University Guro Hospital, Seoul, Korea; ⁷UOC Oncoemetalogia, Università degli Studi della Campania, Naples, Italy; ⁸Centre Eugene Marquis Centre Regional de Lutte Contre Le Cancer, Rennes, France; ⁹Genentech, Inc., South San Francisco, CA; ¹⁰Roche Pharmaceuticals, Mississauga, ON, Canada; ¹¹Roche Products Ltd., Welwyn Garden City, United Kingdom; ¹²Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia and ¹³Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea.

Body: Chemotherapy is the mainstay of treatment for triple-negative breast cancer (TNBC), but taxane resistance remains problematic. TNBC tumors often demonstrate upregulation of the MAPK pathway and increased sensitivity to MEK inhibition. We hypothesized that adding a potent and selective MEK inhibitor such as cobimetinib (C) to paclitaxel (P) would modulate intrinsic taxane resistance and improve progression-free survival (PFS). COLET (NCT02322814) is a phase 2 study with multiple cohorts, including an open-label safety run-in stage and a randomized, placebo (pbo)-controlled stage. Cohort I evaluated C + P vs pbo + P. Cohorts II and III are evaluating triplet combinations of C plus the anti–PD-L1 agent atezolizumab with either P or nab-P. For Cohort I, 90 patients (pts) with previously untreated locally advanced or metastatic TNBC were randomly assigned 1:1 between August 27, 2015, and October 31, 2016, to receive P 80 mg/m² intravenously on days 1, 8, and 15 and either C 60 mg or pbo orally once daily on days 3 through 23 of each 28-day cycle until disease progression or unacceptable toxicity. The primary endpoint was investigator-assessed PFS per Response Evaluation Criteria in Solid Tumors, version 1.1. Secondary endpoints included safety, confirmed and unconfirmed overall response rate (ORR), duration of response, and overall survival. In the primary analysis for the Cohort I randomized stage only, median follow-up was 8 months. All pts were women; 61% had prior adjuvant or neoadjuvant taxane for TNBC. The safety profile of C + P was consistent with that known for the individual drugs, though gastrointestinal and dermatologic adverse events were more frequent than previously reported for C or P alone. Efficacy outcomes in the overall study population are summarized in the table. Median PFS was 5.5 months for C + P vs 3.8 months for pbo + P (HR, 0.73; 95% CI, 0.43–1.24; P = 0.2). Efficacy will also be evaluated in patient subgroups defined by gene expression subtypes, genetic alterations, and PD-L1 expression. COLET is the first randomized, pbo-controlled study to evaluate C + P as first-line treatment for locally advanced or metastatic TNBC. Consistent with the hypothesis that C modulates intrinsic taxane resistance, both median PFS and ORR were increased with the addition of C to standard treatment with P.

**Efficacy Outcomes**

<table>
<thead>
<tr>
<th></th>
<th>C + P (n = 47)</th>
<th>Pbo + P (n = 43)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Duration of follow-up (months)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>8.4 (3.7)</td>
<td>7.8 (3.4)</td>
</tr>
<tr>
<td>Min, max</td>
<td>1.6, 16.8</td>
<td>0.7, 15.7</td>
</tr>
<tr>
<td><strong>PFS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFS events, n (%)</td>
<td>31 (66.0)</td>
<td>31 (72.1)</td>
</tr>
<tr>
<td>Median, months (95% CI)</td>
<td>5.5 (4.2–7.4)</td>
<td>3.8 (1.9–7.2)</td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>0.73 (0.43–1.24)</td>
<td></td>
</tr>
<tr>
<td><strong>Best response</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Confirmed ORR, n (%)</td>
<td>18 (38.3)</td>
<td>9 (20.9)</td>
</tr>
<tr>
<td>Response Type</td>
<td>N (%)</td>
<td>N (%)</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>Complete response, n (%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Partial response, n (%)</td>
<td>18 (38.3)</td>
<td>9 (20.9)</td>
</tr>
<tr>
<td>Stable disease, n (%)</td>
<td>18 (38.3)</td>
<td>16 (37.2)</td>
</tr>
<tr>
<td>Progressive disease, n (%)</td>
<td>8 (17.0)</td>
<td>17 (39.5)</td>
</tr>
<tr>
<td>Unknown, n (%)</td>
<td>3 (6.4)</td>
<td>1 (2.3)</td>
</tr>
</tbody>
</table>

CI, confidence interval; HR, hazard ratio; ORR, overall response rate; PFS, progression-free survival; SD, standard deviation.
Title: Efficacy and safety of abemaciclib in patients with liver metastases in the MONARCH 1, 2, and 3 studies

Angelo Di Leo¹, Maura Dickler², George W Sledge³, Masakazu Toi⁴, Tammy Forrester⁵, Shivani Nanda⁵, Andrew Koustenis⁵, Nawel Bourayou⁶ and Stephen Johnston⁷. ¹Nuovo Ospedale di Prato S. Stefano – Istituto Toscano Tumori, Prato, Italy; ²Memorial Sloan Kettering Cancer Center, New York, NY; ³Stanford University School of Medicine, Stanford, CA; ⁴Graduate School of Medicine, Kyoto University, Kyoto, Japan; ⁵Eli Lilly and Company, Indianapolis, IN; ⁶Eli Lilly and Company, Paris, France and ⁷The Royal Marsden NHS Foundation Trust, London, United Kingdom.

Body: Background:
Abemaciclib is an oral, selective inhibitor of cyclin-dependent kinases 4 & 6 that is dosed on a twice daily continuous schedule. In patients (pts) with hormone receptor-positive (HR+), human epidermal growth factor receptor 2-negative (HER2-) advanced breast cancer (ABC), abemaciclib has demonstrated clinical efficacy with a tolerable safety profile when administered as monotherapy in MONARCH 1 (NCT02102490), in combination with fulvestrant in MONARCH 2 (NCT02107703), and in combination with non-steroidal aromatase inhibitors (NSAI) in MONARCH 3 (NCT02246621). Inducing tumor response and delaying disease progression is of critical need in pts with liver metastases (mets).

Methods:
An exploratory subgroup analysis was conducted in pts with liver mets at baseline across the MONARCH 1, 2, and 3 studies. All pts had HR+, HER2- ABC. The primary endpoint of MONARCH 1 was objective response rate (ORR), and the primary endpoint of MONARCH 2 and 3 was investigator-assessed progression-free survival (PFS). Analysis methods for these endpoints were previously described. Key enrollment criteria and dosing information are listed in Table 1.

Results:
Efficacy results of pts with liver mets are described in Table 2. The most frequent adverse events observed in pts with liver mets in MONARCH 1 were diarrhea, nausea, and fatigue and in the abemaciclib arms of MONARCH 2 and 3 were diarrhea, neutropenia, and nausea.

Table 1. Eligibility criteria and dosing information for the MONARCH 1, 2, and 3 studies

<table>
<thead>
<tr>
<th>Key enrollment criteria</th>
<th>MONARCH 1</th>
<th>MONARCH 2</th>
<th>MONARCH 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior endocrine therapy</td>
<td>Progressed on or after ET</td>
<td>Progressed while receiving adjuvant or first-line ET, or ≤ 12 months from the end of adjuvant ET</td>
<td>ET naïve or disease relapse &gt;12 months after (neo)adjuvant ET</td>
</tr>
<tr>
<td>Chemotherapy regimens in advanced setting</td>
<td>1 or 2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Visceral crisis</td>
<td>No restriction</td>
<td>Not permitted</td>
<td>Not permitted</td>
</tr>
</tbody>
</table>

**Dose and Schedule**

| abemaciclib | 200 mg, twice daily, continuous | 150 mg¹, twice daily, continuous | 150 mg, twice daily, continuous |
| fulvestrant | - | 500 mg, per label | - |
| anastrozole² | - | - | 1 mg, daily |
| letrozole² | - | - | 2.5 mg, daily |

¹post-amendment; ²physician's choice of NSAI (anastrozole or letrozole); ET: endocrine therapy
Table 2. PFS and response rates of pts with liver mets in MONARCH 1, 2, and 3

<table>
<thead>
<tr>
<th></th>
<th>MONARCH 1</th>
<th>MONARCH 2</th>
<th>MONARCH 2</th>
<th>MONARCH 3</th>
<th>MONARCH 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>abemaciclib arm</td>
<td>placebo arm</td>
<td>abemaciclib arm</td>
<td>placebo arm</td>
<td>abemaciclib arm</td>
</tr>
<tr>
<td>Pts with liver mets, n</td>
<td>93</td>
<td>115</td>
<td>59</td>
<td>48</td>
<td>30</td>
</tr>
<tr>
<td>PFS, HR (95% CI)</td>
<td>N/A</td>
<td>.45 (.31, .64)</td>
<td>.47 (.25, .87)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median PFS, months</td>
<td>5.6</td>
<td>11.6</td>
<td>3.1</td>
<td>15.0</td>
<td>7.2</td>
</tr>
<tr>
<td>ORR, n (%)</td>
<td>20 (21.5)</td>
<td>54 (47.0)</td>
<td>9 (15.3)</td>
<td>26 (54.2)</td>
<td>6 (20.0)</td>
</tr>
<tr>
<td>CBR, n (%)</td>
<td>39 (41.9)</td>
<td>77 (67.0)</td>
<td>21 (35.6)</td>
<td>32 (66.7)</td>
<td>12 (40.0)</td>
</tr>
</tbody>
</table>

CBR: clinical benefit rate (complete response [CR] + partial response [PR] + stable disease ≥6 months); HR: hazard ratio; ORR: objective response rate (CR+PR); PFS: progression-free survival; pts: patients

Conclusions:
The results suggest that the combination of abemaciclib and endocrine therapy was an effective treatment option in pts with liver metastases, a population deriving modest benefit from single-agent endocrine therapy. Tolerability results were generally consistent with the safety populations previously reported for each study.
Title: Palbociclib (PAL) + letrozole (LET) as first-line therapy in estrogen receptor–positive (ER+)/human epidermal growth factor receptor 2–negative (HER2−) advanced breast cancer (ABC): Efficacy and safety updates with longer follow-up across patient subgroups

Hope S Rugo1, Richard S Finn10, Veronique Dieras2, Johannes Ettl3, Oleg Lipatov4, Anil Joy5, Nadia Harbeck6, Aurelio Castrellon7, Dongrui R Lu8, Ave Mori8, Eric R Gauthier9, Cynthia Huang8, Karen A Gelmon9 and Dennis J Slamon10. 1University of California San Francisco Comprehensive Cancer Center, San Francisco, CA; 2Institut Curie and Center Eugene Marquis Rennes, Paris, France; 3Frauenklinik und Poliklinik Klinikum rechts der Isar, Technische Universität München, Munich, Germany; 4Republican Clinical Oncology Dispensary, Ufa, Russian Federation; 5Cross Cancer Institute, University of Alberta, Edmonton, Canada; 6Brustzentrum der Universität München, Munich, Germany; 7Memorial Cancer Institute, Pembroke Pines, FL; 8Pfizer, Inc.; 9British Columbia Cancer Agency, Canada and 10David Geffen School of Medicine at UCLA.

Body: BACKGROUND: Endocrine therapy (ET) has been the primary first-line (1L) therapy for ER+ ABC. In the PALOMA-2 study (NCT01740427), PAL (P)+LET (L) significantly prolonged progression-free survival (PFS; HR=0.58, P<.001) after a median 23 mo follow-up (FU) (Finn et al. NEJM 2016). Here we report more mature PFS overall and in subgroups, with (w/) longer FU. The study is ongoing for overall survival FU.

METHODS: Postmenopausal pts w/ ER+/HER2- ABC and no prior systemic therapy in the ABC setting were randomized 2:1 to P (125 mg/d) + L (2.5 mg QD) or placebo (PBO) + L. Key endpoints: investigator-assessed PFS and safety. Median PFS (mPFS) was estimated (intent-to-treat population).

RESULTS: 666 pts (444, P+L; 222, PBO+L) were enrolled. Arms were well balanced: visceral (48%)/nonvisceral (52%) disease and prior ET (56%)/no prior ET (44%). After a median FU of 38 mo w/ P+L and 37 mo w/ PBO+L, mPFS was 27.6 and 14.5 mo, respectively, in the overall population (HR=0.56, P<.0001; Table). The study is ongoing for overall survival FU.

RESULTS: 666 pts (444, P+L; 222, PBO+L) were enrolled. Arms were well balanced: visceral (48%)/nonvisceral (52%) disease and prior ET (56%)/no prior ET (44%). After a median FU of 38 mo w/ P+L and 37 mo w/ PBO+L, mPFS was 27.6 and 14.5 mo, respectively, in the overall population (HR=0.56, P<.0001; Table).

TABLE. mPFS overall and by relevant subgroups

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>P+L</th>
<th>PBO+L</th>
<th>P+L vs PBO+L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>27.6 (22.4–30.3)</td>
<td>14.5 (12.3–17.1)</td>
<td>0.56 (0.46–0.69)</td>
</tr>
<tr>
<td>Measurable disease</td>
<td>23.7 (19.3–27.6)</td>
<td>14.5 (12.3–18.5)</td>
<td>0.63 (0.50–0.79)</td>
</tr>
<tr>
<td>Nonmeasurable disease</td>
<td>36.2 (27.6–NE)</td>
<td>16.5 (8.3–19.6)</td>
<td>0.39 (0.25–0.60)</td>
</tr>
<tr>
<td>Visceral</td>
<td>19.3 (16.4–24.2)</td>
<td>12.3 (8.4–16.4)</td>
<td>0.62 (0.47–0.81)</td>
</tr>
<tr>
<td>Nonvisceral</td>
<td>35.9 (27.7–NE)</td>
<td>17.0 (13.8–24.8)</td>
<td>0.50 (0.37–0.67)</td>
</tr>
<tr>
<td>Bone only†</td>
<td>36.2 (27.6–NE)</td>
<td>11.2 (8.2–22.0)</td>
<td>0.41 (0.26–0.63)</td>
</tr>
<tr>
<td>Not bone only</td>
<td>24.2 (19.4–27.7)</td>
<td>14.5 (12.9–18.5)</td>
<td>0.62 (0.50–0.78)</td>
</tr>
<tr>
<td>De novo metastatic</td>
<td>27.9 (22.1–33.4)</td>
<td>22.0 (13.9–27.4)</td>
<td>0.61 (0.44–0.85)</td>
</tr>
<tr>
<td>Prior ET</td>
<td>24.2 (18.8–27.6)</td>
<td>11.2 (8.4–14.5)</td>
<td>0.54 (0.42–0.71)</td>
</tr>
<tr>
<td>No prior ET</td>
<td>30.3 (24.5–35.7)</td>
<td>21.9 (15.9–27.4)</td>
<td>0.59 (0.43–0.80)</td>
</tr>
<tr>
<td>Nonvisceral</td>
<td>36.2 (27.9–NE)</td>
<td>27.6 (19.1–35.6)</td>
<td>0.59 (0.38–0.92)</td>
</tr>
<tr>
<td>Visceral</td>
<td>23.7 (16.8–30.3)</td>
<td>13.9 (10.2–22.2)</td>
<td>0.55 (0.36–0.85)</td>
</tr>
<tr>
<td>Disease sites</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>30.4 (24.8–NE)</td>
<td>16.5 (11.0–22.1)</td>
<td>0.52 (0.36–0.75)</td>
</tr>
<tr>
<td>2</td>
<td>28.1 (19.4–NE)</td>
<td>16.3 (11.0–27.4)</td>
<td>0.57 (0.37–0.89)</td>
</tr>
<tr>
<td>3</td>
<td>23.7 (19.2–27.6)</td>
<td>13.8 (8.8–17.0)</td>
<td>0.61 (0.46–0.82)</td>
</tr>
</tbody>
</table>
All subgroups benefited from addition of P to L. Notably, pts w/ low disease burden (bone only, nonvisceral disease, few disease sites) derived significant PFS benefit, including those w/ both nonvisceral disease and no prior ET (mPFS, 36.2 vs 27.6 mo; HR=0.59, P<.01). Importantly, median time from randomization to start of 2nd subsequent systemic anticancer therapy was 39 vs 29 mo for P+L vs PBO+L (HR=0.72, P<.005). There were no new safety signals w/ longer FU.

**CONCLUSIONS:** This is the longest FU of a phase 3 study of a cyclin-dependent kinase 4/6 inhibitor for ABC. P+L continues to consistently improve PFS vs PBO+L across all subgroups while toxicity remains manageable; notably P+L delays time to starting 2nd subsequent anticancer therapies by 10 mo. Pts w/ low disease burden or sensitivity to ET alone had PFS >3 y (significant vs PBO+L), demonstrating the clinical benefit of P+ET. These data confirm P+L should be a 1L therapy option for pts w/ HR+/HER2-ABC.

**Funding:** Pfizer
Title: Phase I/Ib study of the SERD LSZ102 alone or in combination with ribociclib in ER+ breast cancer

Body: Background: LSZ102 is an orally bioavailable selective estrogen receptor degrader (SERD) that inhibits estrogen receptor (ER) gene transcription, induces receptor degradation, and blocks ER-dependent cell growth in preclinical models. This Phase I/Ib, open-label study is evaluating LSZ102 as a single agent and in combination with the CDK4/6 inhibitor ribociclib (LEE011) or the PI3K inhibitor alpelisib (BYL719) in patients (pts) with locally advanced/metastatic ER-positive (ER+) breast cancer (BC).

Methods: The primary objective is to characterize the safety and tolerability, and identify a recommended dose and regimen of LSZ102 alone (Arm A) or in combination with ribociclib (Arm B) or alpelisib (Arm C). Secondary objectives include evaluation of preliminary antitumor activity and pharmacokinetics (PK). Eligible pts (aged ≥18 yrs; ECOG PS 0-1) have histologically confirmed ER+ BC that has progressed after endocrine therapy.

Results: As of March 14, 2017, dose escalation evaluating 16 pts in Arm A (LSZ102 200 mg [n=4], 400 mg [n=6], and 600 mg [n=6]) had completed (median age 57.5 yrs; 81% ECOG PS 0; 63% received prior fulvestrant). Five pts (median age 59.0 yrs; 80% ECOG PS 0; 60% received prior fulvestrant) had enrolled in the first cohort of Arm B (LSZ102 200 mg QD + ribociclib 300 mg 3 weeks on/1 week off) with evaluation ongoing. Arm C (LSZ102 + alpelisib) had yet to open. As of March 14, 2017, 9/16 (56%) pts in Arm A had discontinued treatment, all due to progressive disease (PD); in Arm B all pts were still receiving treatment. There were no dose-limiting toxicities in either arm at the dose levels evaluated; dose escalation is ongoing. The most common drug-related adverse events (AEs) were diarrhea (Grade [Gr] 1: 7/16; Gr 2: 2/16 pts), nausea (Gr 1: 6/16; Gr 2: 2/16 pts), and vomiting (Gr 1: 3/16 pts) in Arm A, and hot flush, nausea, vaginal discharge (all Gr 1: 2/5 pts), thrombocytopenia (Gr 1: 1/5; Gr 2: 1/5 pts), and neutropenia (Gr 2: 1/5, Gr 3: 1/5 pts) in Arm B. There were no drug-related Gr 3/4 AEs reported in Arm A; in Arm B, Gr 3 neutropenia, leukopenia, and lymphopenia each occurred in 1/5 pts. Preliminary PK assessment showed single-agent LSZ102 exposure increased dose-proportionally from 200 to 600 mg QD. In combination with ribociclib, exposures were consistent with those of the single agent at the same dose. In Arm A, preliminary evidence of antitumor activity was observed. Efficacy data for Arms B and C were not available as of March 14, 2017. One pt, whose tumor harbored an ESR1 D538G mutation, had been treated with multiple prior therapies in the metastatic setting, including letrozole, exemestane, tamoxifen, exemestane + everolimus, and anastrozole, as well as fulvestrant for 120 days prior to PD, and letrozole + palbociclib for 94 days prior to PD. As of March 14, 2017, this pt had been on LSZ102 treatment (400 mg QD) for 167 days, with a best response of stable disease (14% reduction in sum of diameter of target lesions).

Conclusions: Oral single-agent LSZ102 appears well-tolerated, with a manageable safety profile. Preliminary data also suggest tolerability when combined with ribociclib. Preliminary evidence of single-agent antitumor activity was seen in heavily pretreated pts with ER+ BC in a post-fulvestrant setting.
Title: Alpelisib plus letrozole in estrogen receptor-Positive (ER+), human epidermal growth factor receptor 2-negative (HER2−) advanced breast cancer (aBC): Safety and preliminary efficacy analysis from a phase 1b trial

Dejan Juric1, Anthony Gonçalves2, Erika Hamilton3, Valentina Boni4, Ingrid A Mayer5, Sabine Turri6, Yingbo Wang7, Florian D Vogl7, Dalila Sellami8 and Mario Campone9. 1Massachusetts General Hospital Cancer Center, Boston, Massachusetts; 2Institut Paoli–Calmettes, Marseille, France; 3Sarah Cannon Research Institute and Tennessee Oncology, Nashville, Tennessee; 4START Madrid-Centro Integral Oncológico Clara Campal Hospital, Madrid, Spain; 5Vanderbilt-Ingram Cancer Center, Nashville, Tennessee; 6Novartis Pharma S.A.S, Rueil-Malmaison, France; 7Novartis Pharma AG, Basel, Switzerland; 8Novartis Pharmaceuticals Corporation, East Hanover, New Jersey and 9Institut de Cancérologie de l'Ouest/René Gauducheau, Saint Herblain, France.

Body: Background
Endocrine therapy is the standard first-line treatment for postmenopausal patients (pts) with ER+, HER2– aBC. However, resistance eventually develops, often through dysregulation of PI3K/AKT/mTOR pathway, specifically mutations in PIK3CA, the gene encoding the p110α subunit of PI3K. The oral, selective PI3K inhibitor alpelisib and letrozole synergistically inhibits tumor growth in preclinical models of ER+ breast cancer. Alpelisib plus letrozole in pts with ER+ aBC is being investigated in arm 2 of a multi-arm, phase 1b study (NCT01872260).

Methods
Postmenopausal women with ER+, HER2− aBC received alpelisib (300 mg QD; continuous, 28 days cycle) plus letrozole (2.5 mg QD; continuous). Primary endpoints were to confirm MTD/RP2D of alpelisib plus letrozole in the escalation phase and to further characterize safety and tolerability in the expansion phase. Secondary and exploratory endpoints included efficacy, pharmacokinetics, and biomarkers.

Results
As of August 19, 2016, 56 pts had received alpelisib plus letrozole: 19 pts were enrolled in the escalation phase (designated here as previously treated group), of which, 95% of pts were previously treated for aBC and 37 pts were enrolled in the expansion phase (designated here as first-line group), of which, 81% of pts were treatment-naïve for aBC. 16 previously treated pts and 11 first-line pts (48% of all pts) have discontinued treatment. Most common reasons for treatment discontinuation in the full population were disease progression (23.2%) and adverse events (AEs) (8.9%). Median duration of exposure of combination (alpelisib plus letrozole) was 23 weeks and 12.7 weeks in previously treated and first-line groups, respectively. Most frequently reported any grade treatment-related AEs (≥20% incidence) in all pts were hyperglycemia (48.2%), diarrhea (48.2%), nausea (33.9%), and decreased appetite (28.6%). Most common, grade 3 or 4 AEs (≥3% incidence) suspected to be treatment-related in all pts included hyperglycemia (17.9%), rash (5.4%), and diarrhea (3.6%). Median progression-free survival was 5.7 months in the previously treated group and was not reached in the first-line group. A summary of best overall response, overall response rate and clinical benefit rate in evaluable pts is shown in the table.

<table>
<thead>
<tr>
<th>Best overall response, n (%)</th>
<th>Alpelisib+Letrozole (Previously Treated group) [N=19]</th>
<th>Alpelisib+Letrozole (First-line group) [N=27]</th>
<th>All subjects (N=46)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confirmed CR</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Confirmed PR</td>
<td>0</td>
<td>4 (14.8)</td>
<td>4 (8.7)</td>
</tr>
<tr>
<td>NCRNPD</td>
<td>6 (31.6)</td>
<td>9 (33.3)</td>
<td>15 (32.6)</td>
</tr>
<tr>
<td>SD</td>
<td>8 (42.1)</td>
<td>9 (33.3)</td>
<td>17 (37.0)</td>
</tr>
<tr>
<td>PD</td>
<td>2 (10.5)</td>
<td>1 (3.7)</td>
<td>3 (6.5)</td>
</tr>
<tr>
<td>Unknown</td>
<td>3 (15.8)</td>
<td>3 (11.1)</td>
<td>6 (13.0)</td>
</tr>
<tr>
<td>ORR (CR+PR), % (95% CI)</td>
<td>0 (0.0-17.6)</td>
<td>14.8 (4.2-33.7)</td>
<td>8.7 (2.4-20.8)</td>
</tr>
</tbody>
</table>
CBR [CR+PR+(SD/NCRNP)], %
(95% CI) 36.8 (16.3-61.6) 70.8 (48.9-87.4) 55.8 (39.9-70.9)

CBR; clinical benefit rate; CI, confidence interval; CR, complete response; NCRNP; Non-CR/Non-PD; ORR; overall response rate; PD; progressive disease; SD, stable disease.

Conclusions
Based on these preliminary data, the combination of alpelisib plus letrozole had manageable safety profile in pts with ER+, HER2- aBC and demonstrated encouraging clinical activity, particularly in the first-line patient population.
**2017 San Antonio Breast Cancer Symposium**

**Publication Number:** P5-21-07

**Title:** Phase II study of eribulin in combination with pertuzumab plus trastuzumab for human epidermal growth factor receptor 2 (HER2)-positive advanced or metastatic breast cancer

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**Body:**

**Background:** Pertuzumab provided overall and progression-free survival (PFS) benefits in HER2-positive metastatic breast cancer patients (pts) in the CLEOPATRA (Clinical evaluation of docetaxel, pertuzumab and trastuzumab) study. However, few studies have described the efficacy of other drugs in combination with pertuzumab plus trastuzumab. Here, we present a pre-specified analysis of eribulin in combination with pertuzumab plus trastuzumab as first- and second-line therapy for advanced or metastatic breast cancer (AMBC) in a multicenter, open-label phase II study (UMIN000012232, JBCRG-M03).

**Methods:** HER2-positive AMBC with no or single prior chemotherapy for AMBC were enrolled. All pts were administered trastuzumab and taxane as adjuvant or first-line chemotherapy. Treatment consisted of eribulin 1.4 mg/m² on days 1 and 8 of a 21-day cycle and trastuzumab (8 mg/kg loading dose, then 6 mg/kg) plus pertuzumab (840 mg/body loading dose, then 420 mg/body) once every 3 weeks, all administered intravenously. The primary endpoint was PFS, and secondary endpoints included overall response rate (ORR) and safety. PFS was determined using Kaplan–Meier analysis. Tumor response was assessed according to RECIST ver. 1.1.

**Results:** Fifty pts were enrolled from November 2013 to April 2016. Forty-nine pts were eligible for safety analysis and the full analysis set (FAS) included 46 pts. The median age was 56 years (23–70), and 8 (16%) and 41 (84%) pts were treated in first- and second-line settings, respectively. Eleven pts (23.9%) were de-novo Stage 4, and 35 pts (76.1%) had progressed in metastatic disease after completion of local therapy. Median PFS was 9.3 months (M) (95% confidence interval [CI]: 6.4–12.3). Table 1 shows the efficacy data for each treatment line and includes ORR, complete response rate (CR), partial response rate (PR), stable disease rate (SD), progressive disease rate (PD), not evaluable rate (NE) and PFS in the FAS. The median relative dose intensities of eribulin, trastuzumab, and pertuzumab were 93.3% (77.0%–100%), 100% (96.0%–100%), and 100% (89.7%–100%), respectively, in the FAS. The grade 3/4 adverse events (AE) were neutropenia in 5 pts (10.2%), including 2 pts (4.1%) with febrile neutropenia; hypertension in 3 pts (6.1%), and other AEs in only one patient. The average of the ejection fraction did not decrease significantly. Symptomatic left ventricular systolic dysfunction was not observed.

**Conclusion:** In pts with HER2-positive AMBC, first- and second-line therapy of eribulin in combination with pertuzumab plus trastuzumab demonstrated substantial antitumor activity with an acceptable safety profile. We are planning a phase III study comparing eribulin with taxanes in combination with pertuzumab plus trastuzumab for the treatment of HER2-positive AMBC.

**Table 1:** Efficacy data for each treatment line

<table>
<thead>
<tr>
<th>Treatment Line</th>
<th>Total (n=46)</th>
<th>First line (n=8)</th>
<th>Second line (n=38)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFS (95% CI), months</td>
<td>9.3 (6.4-12.3)</td>
<td>20.8 (2.8-38.7)</td>
<td>8.7 (7.2-10.2)</td>
</tr>
<tr>
<td>ORR (%)</td>
<td>28 (60.9)</td>
<td>7 (87.5)</td>
<td>21 (55.3)</td>
</tr>
<tr>
<td>CR (%)</td>
<td>8 (17.4)</td>
<td>3 (37.5)</td>
<td>5 (13.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>PR (%)</td>
<td>20 (43.5)</td>
<td>4 (50.0)</td>
<td>16 (42.1)</td>
</tr>
<tr>
<td>SD (%)</td>
<td>11 (23.9)</td>
<td>1 (12.5)</td>
<td>10 (26.3)</td>
</tr>
<tr>
<td>PD (%)</td>
<td>5 (10.9)</td>
<td>0</td>
<td>5 (13.2)</td>
</tr>
<tr>
<td>NE (%)</td>
<td>2 (4.3)</td>
<td>0</td>
<td>2 (5.3)</td>
</tr>
</tbody>
</table>
Title: Tolerability of the combination of lapatinib and trastuzumab in older patients with HER2 positive metastatic breast cancer


Body: Background: Older adults are less likely to be included in clinical trials leading to the approval of novel cancer treatments. The Institute of Medicine and ASCO have identified therapeutic phase II trials as a key research priority to increase the evidence base for older adults with cancer. While targeted therapies may represent a less toxic option for older patients, few trials have studied their tolerability and efficacy in older adults. Here, we present a phase II study (NCT01273610) of the combination of trastuzumab and lapatinib in older patients with HER2+ metastatic breast cancer (MBC), incorporating geriatric oncology principles in the study design.

Methods: Patients age ≥ 60 years with MBC and any number of prior chemotherapy (CT) lines received trastuzumab (either 4mg/kg loading dose followed by 2mg/kg weekly or 8mg/kg followed by 6mg/kg q/3 weeks) plus lapatinib 1000 mg/m$^2$ daily in 21-day cycles. Patients completed a pre-treatment geriatric assessment including measures of function, comorbidity, cognition, nutrition, and psychosocial status. A toxicity risk score developed for older adults receiving cytotoxic CT was calculated for each patient (Hurria et al. JCO 2011 & 2016). Relationships between tolerability (dose reductions and grade (G) ≥ 3 toxicity attributed to treatment) and risk score analyzed using a log$_2$ transformation were assessed using generalized linear models, Student's t tests, and Fisher's exact test. Response rate (RR) and progression free survival (PFS) were evaluated.

Results: 40 patients (mean age 72 [60-92]) were accrued from 04/11 to 05/15. 25% (n = 10) were ≥ 75 years of age. 65% of patients (n = 26) had HR+ tumors and 35% (n = 14) were receiving ≥ 3rd line treatment. Median number of cycles was 4 (0-28). RR was 23% (n = 9, 95% CI 11-38%; 1 complete, 8 partial). 23% (n = 9) achieved stable disease. PFS was 2.7 months (95% CI 2.5-12). Based on the toxicity risk score, 21% (n = 8), 54% (n = 21), and 26% (n = 10) were at low, intermediate, and high risk. 70% (n = 28) of patients had G ≥ 2 toxicities and 20% (n = 8) G ≥ 3 toxicities. G 2 and 3 diarrhea occurred in 28% (n = 11) and 5% (n = 2) respectively. 5% (n = 2) were hospitalized due to treatment-related toxicity. No G ≥ 3 cardiac toxicities were observed. 23% of patients (n = 9) had treatment delays, and 43% (n = 17) required a lapatinib dose reduction. The mean toxicity risk score was higher in patients who required dose reductions (Student's t: p = 0.02). No statistically significant relationship was found between toxicity risk scores and the presence of G ≥ 3 treatment toxicity (logistic regression: OR = 3.08, 95% CI [0.54, 21.2], p = 0.22).

Conclusions: Among older patients with MBC (79% at intermediate or high risk of G ≥ 3 cytotoxic CT toxicity), trastuzumab and lapatinib were well tolerated, with only 20% experiencing G3 toxicities. The toxicity risk score was not found to be significantly related with treatment toxicity, which may be explained by the very low incidence of G3 events. Patients with a low toxicity risk score were not likely to require a lapatinib dose reduction.
Breast Cancer registries can help to understand how patient groups are treated outside clinical trials and what outcome is to expected for specific patient groups and therapy lines, which are not included into clinical trials. Here we present overall survival data according to therapy lines, patient and tumor characteristics.

Methods

The PRAEGNANT metastatic breast cancer registry (NCT02338167) is a prospective registry for breast cancer patients with focus on molecular biomarkers. Patients of all therapy lines with any kind of treatment are eligible for this registry. Collected data comprises therapies, adverse events, quality of life and other patient reported outcomes. Here we report on the comparison of overall survival data for different patient groups. For that analysis was restricted to patients included in the first therapy line for subgroup comparisons. Only for the analysis of the effect of therapy line on overall survival the complete dataset was used.

Results

A total of 1854 patients took part in this analysis. Of those 1016 were included first line, and 340, 213 and 285 patients 2nd, 3rd lin4 and 4th line or higher respectively. A total of 339 deaths were observed. Two year survival rates (2Y-OS) for these groups were 75%, 65%, 57% and 36% (p(log-rank)<0.001). All further analyses were done only for the subset of first line treated patients with a total of 127 events. HER2 positive patients had the highest 2Y-OS rate of 84% and TNBC had the worst with 60% (see figure 1). Absolute numbers of deaths for TNBC, luminal A like, luminal B like and HER2 posives were 21, 48, 25, and 21. According to metastastic pattern the following survival rates were seen: brain (48%), other locations (69%), visceral (78%) and bone only (81%). ECOG was another factor that had a large influence on OS with ECOG 0: 83%, ECOG 1: 67% and ECOG 2+: 63% 2Y-OS. Age, BMI, number of concomitant diseases, and primary metastatic status did not have an influence on 2Y-OS.

Conclusion

This breast registry included patients over all therapy lines however mainly during the 1st line of therapy. Simple patient and tumor characteristics can classify patients into patients with differently favourable prognosis. Patients with HER2 positive disease had the best overall survival, while TNBC and luminal B like patients had the worst prognosis. As most of the patients were luminal A like 43% of all deaths occured within the group of luminal A like patients, which will need further focus for therapy development in the future.
Title: Phase 2 study and correlative analyses of ruxolitinib, a selective JAK1/2 inhibitor, in patients with metastatic, triple-negative breast cancer

Daniel G Stover¹, Carlos R Gil Del Alcazar¹, Sara M Tolaney¹, Aditya Bardia², Hao Guo¹, Justin M Balko³, Beth A Overmoyer¹, Rebecca S Gelman¹, Max Lloyd¹, Vivian Wang¹, Jane E Brock¹, Eric P Winer¹, Kornelia Polyak¹ and Nancy U Lin¹. ¹Dana-Farber Cancer Institute, Boston, MA; ²Massachusetts General Hospital Cancer Center, Boston, MA and ³Vanderbilt University Medical Center, Nashville, TN.

Body: Background: Preclinical data supports a role for the IL-6/JAK2/STAT3 signaling pathway in breast cancer (BC).
Ruxolitinib is an orally bioavailable receptor tyrosine inhibitor targeting JAK1 and JAK2. We evaluated the safety and efficacy of ruxolitinib in patients with metastatic BC and performed correlative analyses.

Methods: This was a non-randomized, phase 2 study of patients with refractory, metastatic, triple-negative BC (TNBC). Patients with inflammatory BC (IBC) of any subtype were also enrolled. The primary endpoint was objective response by RECIST 1.1. Secondary endpoints included progression-free survival (PFS), overall survival (OS), and toxicity. The study was designed to enroll only patients whose archival tumor tissue was pSTAT3 moderately to strongly positive in the tumor epithelial cells by central immunohistochemistry (IHC). 16 patients underwent pre-treatment biopsy, of whom 4 also had a second biopsy prior to cycle 2. Biopsy samples and paired primary tumor samples (when available) were subjected to multi-color immunofluorescence and/or immune-FISH for leukocyte markers, pSTAT3, and JAK2. RNA sequencing was performed on available on-study frozen biopsy specimens. 17 patients had plasma collected with cell-free DNA (cfDNA) extracted and subjected to low coverage whole-genome sequencing.

Results: Of 217 patients who consented to archival tumor testing, T-score for pSTAT3 was ‘high’ (>5) in 69 patients (31.8%), demonstrating frequent activation of the JAK/STAT pathway in metastatic TNBC or IBC. 23 pSTAT3 high patients were enrolled. Ruxolitinib was generally well-tolerated. The most commonly observed adverse events (any grade) were anemia, neutropenia, thrombocytopenia, constipation, nausea, and increased AST/ALT. Grade 3 or higher toxicities were uncommon. No objective responses were seen among 21 evaluable patients, therefore the study was closed to accrual based on study design. Intensive correlative analyses revealed important insights regarding ruxolitinib effects. Pharmacodynamic analyses of baseline versus cycle 2 biopsies demonstrate downregulation of JAK2 target genes, STAT3 signatures, and JAK/STAT gene ontology gene sets, suggesting on-target activity. There was evidence of immune microenvironment modulation: gene set enrichment analysis implicated reduced macrophage/myeloid phenotypes after treatment and CIBERSORT analysis of inferred immune cell subsets demonstrated reduced monocyte/macrophage proportion after treatment (t-test p=0.013). Multi-color immunofluorescence analyses of immune microenvironment are ongoing and will be reported. 17 patients underwent cfDNA analysis with 8 patients (47%) demonstrating gain or amplification of JAK2.

Conclusions: Ruxolitinib, as a single agent, did not meet the primary efficacy endpoint in this refractory patient population. Correlative studies demonstrate evidence of on-target activity and immune microenvironment modulation. Frequent JAK/STAT pathway activation and JAK2 locus chromosomal gains in this cohort suggest that the JAK/STAT pathway remains a potential therapeutic target in BC.
2017 San Antonio Breast Cancer Symposium

Publication Number: P5-21-11

Title: Benefit from palbociclib and fulvestrant based on previous fulvestrant and/or everolimus treatment. Based on a cohort of over 200 patients treated in a French compassionate program

Monica Arnedos¹, Pauline Rusquec², Magali Morelle³, Coriolan Lebreton⁴, Emmanuelle Jacquet⁵, George Emile⁶, Jonathan Aires⁷, Marc Debled⁸, Jean-Sebastien Frenel², Paule Augereau⁷, Bianca Cheaib¹, Christelle Levy⁵ and Thomas Bachelot³. ¹Gustave Roussy Cancer Campus, Villejuif, France; ²Institut Cancerologie Ouest, Nantes, France; ³Centre Leon Bedard, Lyon, France; ⁴Institut Bergonie, Bordeaux, France; ⁵Centre Francois Baclesse, Caen, France; ⁶Hospital San Pedro de Alcántara, Caceres, Spain and ⁷Institut Cancerologie Ouest, Angers, France.

Body: Background: CDK4/6 inhibitors have been approved in the recent years for the treatment of advanced hormone receptor-positive breast cancer. For patients with resistance to previous endocrine therapy, the approval is based on the results of the PALOMA-3 trial testing palbociclib in addition to fulvestrant observing a progression-free survival (PFS) of 9.2 months. Nevertheless, in this study no previous treatment with fulvestrant was allowed and no information had been reported of efficacy after everolimus administration.

Patients and methods: We collected information from patients treated with palbociclib + fulvestrant in the context of a French compassionate access. We aimed at determining the benefit of this treatment in a real population to provide information about PFS in non-selected patients as well as efficacy of palbociclib and fulvestrant in patients previously treated with fulvestrant and/or everolimus. Median PFS were assessed by Kaplan-Meier survival analysis and compared with log-rank test.

Results: 206 patients were identified from 5 institutions. Mean age at treatment was 61 years (range 28 – 85). 55% presented with visceral disease. Lines at where palbociclib + fulvestrant treatment was administered were as follows: 1% 1st line, 8.3% 2nd line, 19.4% 3rd line, 13.6% 4th line, 10.2% 5th lines and the remaining 47.6% had received ≥ 6 lines (median: 5 lines, range 1 to 15).

A total of 48% patients had previously been treated with fulvestrant. In a subsample of patient where the information was available (n=146), 67.8% patients had received everolimus in combination with endocrine therapy before palbociclib administration.

A total of 77 patients were still on treatment. Median PFS on fulvestrant-palbociclib treatment at the date of data cut-off was of 5.46 months (95% CI; 4.6 to 6.6 months). In a univariate analysis, there were no significant differences in median PFS for patients treated or not with previous fulvestrant, suggesting a potential effect of palbociclib to recover sensitivity to fulvestrant (4.7 months for previous fulvestrant treatment [95% CI 4.07 - 6.3 months] vs 6.1 months for no previous fulvestrant [95% CI; 6.3 - 8.02 months], p=0.3559).

Similarly, in the subsample of n=146 patients where information about previous everolimus treatment was available at data cut-off, benefit from palbociclib-fulvestrant was not affected by previous everolimus treatment (median PFS 4.8 months for previously treated [95% CI; 4.01 - 7.8 months] vs 5.4 months for the untreated everolimus group [95% CI; 4.07 - 9.59 months], p=0.374).

Conclusions: Fulvestrant-palbociclib in the real life is associated with a median PFS of 5.5 months, which is below the results provided in the PALOMA-3 trial, reflecting a much more advanced population. Importantly, neither previous everolimus treatment nor fulvestrant therapy affected benefit from fulvestrant-palbociclib in this population in univariate analyses suggesting a potential recovery of fulvestrant sensitivity with CDK4/6 inhibition. Results from multivariate analyses and more detailed information about patients’ characteristics and benefit from previous therapies will be provided.
**2017 San Antonio Breast Cancer Symposium**

**Publication Number:** P5-21-12

**Title:** Tolerability of olaparib monotherapy versus chemotherapy in patients with HER2-negative metastatic breast cancer and a germline *BRCA* mutation: OlympiAD

Susan M Domchek1, Mark Robson2, Seock-Ah Im3, Elżbieta Senkus4, Binghe Xu5, Norikazu Masuda6, Suzette Delaloge7, Wei Li8, Anne Armstrong9, Pierfranco Conte10, Wendy Bannister11, Carsten Goessl12, Sarah Runswick13, Saurabh Goel12 and Nadine Tung14. 1Basser Center, University of Pennsylvania, Philadelphia, PA; 2Memorial Sloan Kettering Cancer Center, NY; 3Seoul National University Hospital, Cancer Research Institute, Seoul National University College of Medicine, Seoul, Korea; 4Medical University of Gdańsk, Gdańsk, Poland; 5National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China; 6National Hospital Organization, Osaka National Hospital, Osaka, Japan; 7Institut Gustave Roussy, Villejuif, France; 8The First Hospital of Jilin University, Changchun, China; 9Christie Hospital NHS Foundation Trust and Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, United Kingdom; 10University of Padova and Istituto Oncologico Veneto IRCCS, Padova, Italy; 11AstraZeneca, Cambridge, United Kingdom; 12AstraZeneca, Gaithersburg; 13AstraZeneca, Macclesfield, United Kingdom and 14Beth Israel Deaconess Medical Center, Dana-Farber Harvard Cancer Center, Boston, MA.

**Body:** Background

The OlympiAD study (NCT02000622) showed a significant progression-free survival benefit for olaparib monotherapy over chemotherapy treatment of physician’s choice (TPC) in patients (pts) with HER2-negative metastatic breast cancer (mBC) and a germline *BRCA* mutation (g*BRCA*m). We examined olaparib tolerability in these pts to further characterize the most common adverse events (AEs).

**Methods**

OlympiAD was a randomized, open-label, Phase III study in g*BRCA*m, HER2-negative mBC pts who had received ≤2 chemotherapy lines in the metastatic setting. Pts were randomized 2:1 to olaparib tablet (300 mg bid) or single-agent TPC (capecitabine, eribulin or vinorelbine). Treatment continued until disease progression or unacceptable toxicity.

**Results**

302 pts were randomized to olaparib (n=205) or TPC (n=97). Six TPC pts declined study treatment due to treatment allocation and were excluded from safety analyses. There were fewer Grade ≥3 AEs with olaparib vs TPC (36.6% vs 50.5%, respectively) and a low rate of discontinuation due to toxicity (4.9% for olaparib vs 7.7% for TPC). Anemia, nausea, vomiting, fatigue and headache were more frequent with olaparib, and neutropenia, decreased white blood cells (WBC) and palmar-plantar erythrodysesthesia (PPE) were more common with TPC. Incidence and outcome for the most common AEs (>20% pts in either arm) are shown in the table. There were no Grade ≥3 nausea or vomiting with olaparib. The first onset of nausea in either arm was typically within the first month of treatment. Prevalence of nausea with olaparib treatment was highest in the first 3 months (~30% of pts), decreasing to ~15% for the remainder of the study period. Grade ≥3 anemia was more frequent in the olaparib versus TPC arms. More olaparib pts required blood transfusion (18.0%) than TPC pts (5.5%), and there was more concomitant use of erythropoietin stimulating agents with olaparib than TPC (5.9% vs 1.0%, respectively). Anemia onset was early, typically in the first 3 months of starting treatment. The prevalence of anemia was relatively stable over time. Grade ≥3 neutropenia was more frequent in pts receiving TPC instead of olaparib. There was a slightly higher incidence of concomitant granulocyte-colony stimulating factor treatment with TPC (8.2%) vs olaparib (5.4%).

<table>
<thead>
<tr>
<th></th>
<th><strong>Olaparib N=205 (%)</strong></th>
<th></th>
<th><strong>TPC N=91 (%)</strong></th>
</tr>
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<tbody>
<tr>
<td>Nausea</td>
<td>58 [0]</td>
<td>1 [2]</td>
<td>0</td>
</tr>
<tr>
<td>Vomiting</td>
<td>30 [0]</td>
<td>1 [2]</td>
<td>0</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>21 [1]</td>
<td>0 [1]</td>
<td>0</td>
</tr>
</tbody>
</table>
### AEs of any cause. MedDRA preferred terms are combined for 1) anemia and 2) neutropenia

<table>
<thead>
<tr>
<th></th>
<th>Count 1</th>
<th>Count 2</th>
<th>Count 3</th>
<th>Count 4</th>
<th>Count 5</th>
<th>Count 6</th>
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</thead>
<tbody>
<tr>
<td>Headache</td>
<td>20 [1]</td>
<td>0 [0]</td>
<td>0</td>
<td>15 [2]</td>
<td>1 [0]</td>
<td>0</td>
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<tr>
<td>PPE</td>
<td>1 [0]</td>
<td>0 [0]</td>
<td>0</td>
<td>21 [2]</td>
<td>8 [6]</td>
<td>1</td>
</tr>
</tbody>
</table>

### Conclusions

Gastrointestinal toxicity in either arm was mostly mild to moderate, and typically reported early after treatment initiation. Anemia was more frequent in olaparib pts but rarely led to treatment cessation. Olaparib tablets were generally well tolerated in HER2-negative mBC pts with a gBRCAm, with a lower rate of Grade ≥3 AEs compared with chemotherapy.
2017 San Antonio Breast Cancer Symposium

Publication Number: P5-21-13

Title: Olaparib monotherapy versus chemotherapy for patients with HER2-negative metastatic breast cancer and a germline BRCA mutation: Asian subgroup analysis from the phase III OlympiAD trial

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Body: Background
In the Phase III OlympiAD trial (NCT02000622, D0819C00003), olaparib (Lynparza™) showed a significant progression-free survival (PFS) improvement compared with chemotherapy treatment of physician's choice (TPC) in patients (pts) with metastatic breast cancer (mBC) and a germline BRCA mutation (gBRCAm) (Robson et al. NEJM 2017). Here, we present data from a subgroup analysis of Asian pts. It is not yet known whether Asian pts, in comparison with the global patient population, may experience instances of differential toxicity with olaparib therapy.

Methods
OlympiAD, an open-label, multicenter, Phase III trial, randomized (2:1) pts with HER2-negative mBC and a gBRCAm to olaparib tablets (300 mg twice daily) or single-agent TPC (21-day cycles of capecitabine, eribulin or vinorelbine). Pts must have received ≤2 lines of chemotherapy for mBC and prior anthracycline and taxane in the adjuvant, neo-adjuvant or metastatic setting. Primary endpoint was PFS by blinded independent central review (BICR). Region (Asia, Europe, North America, South America) was a pre-defined subgroup for PFS.

Results
The Asian subgroup analysis included pts randomized at centers in China, Japan, Korea and Taiwan. Of 87 Asian pts randomized (median age 46 years), 86 received study treatment (n=59, olaparib; n=27, TPC). In the olaparib group, 29/59 (49%) had estrogen receptor positive (ER+) and/or progesterone receptor positive (PR+) tumors, and 30/59 (51%) had triple negative breast cancer (TNBC). In the TPC group, 13/28 (46%) had ER+/PR+ tumors and 15/28 (54%) had TNBC. The primary endpoint, PFS by BICR, favored olaparib with a hazard ratio (HR) of 0.53 (95% confidence interval [CI] 0.29–0.97; median 5.7 vs 4.2 months; 77% maturity), and was supported by investigator-assessed PFS (HR 0.29, 95% CI 0.16–0.55). In the overall OlympiAD study population (N=302), the PFS by BICR favored olaparib with a HR of 0.58 (95% CI 0.43–0.80; P=0.0009). Within the Asian subgroup, objective response rate (ORR) (RECIST) was 64% for olaparib versus 38% for the TPC group. Time to second progression, PFS2, was longer for pts receiving olaparib versus TPC (HR 0.43, 95% CI 0.22–0.84; 57% maturity). Grade ≥3 adverse events (AEs) occurred in 46% and 59% of pts receiving olaparib and TPC, respectively. The most common grade ≥3 AE was anemia (olaparib, 20%; TPC, 15%). In both treatment groups, 7% of pts discontinued study treatment due to AEs (n=4, olaparib; n=2, TPC). The tolerability profile of olaparib between the subgroup of Asian pts and the overall OlympiAD population will be examined in our data presentation.

Conclusion
Olaparib demonstrated an efficacy benefit compared with TPC in pts with HER2-negative mBC and a gBRCAm in this subgroup analysis of Asian pts from the Phase III OlympiAD trial. Discontinuation rates due to toxicity were low, highlighting that olaparib was generally well-tolerated. The efficacy of olaparib within the subgroup of Asian pts was consistent with that shown for the full OlympiAD dataset; consistent hazard ratios were shown in favor of olaparib using the primary endpoint of PFS by BICR, and for the key secondary endpoints of PFS by investigator assessment, PFS2, and ORR.
Title: Long-term survival in HER2-positive metastatic breast cancer treated with first-line trastuzumab: Real-life results from the Curie ESME database

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Body: Background
About 20% of breast cancer (BC) patients will develop distant metastases. HER2-positive (HER2+) disease is associated with poorer prognosis. However, outcome of HER2+ BC patients has dramatically improved since the use of trastuzumab. Although most HER2+ metastatic BC (MBC) patients will eventually develop progressive disease within one year of trastuzumab-based treatment, some are long-term responders (LTR). Few data on these patients are available.

Methods
The primary objective was to assess overall survival (OS) of HER2+ MBC patients treated with trastuzumab as 1st-line at the Institut Curie, based on institution-specific data included in the French Epidemiological Strategy and Medical Economics (ESME) program for MBC patients established in 2014 by Unicancer. Long-term responders (LTR) were defined as having a non-progressive disease for at least 2 years under 1st-line trastuzumab. Secondary objectives included progression-free survival (PFS), disease-free interval (DFI), prediction factors for LTR status by univariate and multivariate analysis: characteristics of patients (age, BMI, menopausal status), of primary tumour (e.g. hormone receptor (HR) status), of treatment (e.g. endocrine therapy, partner for trastuzumab combination), number and type of metastases.

Results
From 2008 to 2014, 2990 MBC patients were identified in the Curie-ESME MBC database. Of these, 460 had HER2+ disease. Sixteen patients with second primary malignancy were excluded. Of the 444 remaining patients, 340 received 1st-line trastuzumab and could be analyzed. With a 42.5-month median follow-up time, the mean age at metastatic diagnosis was 55.7 years, 59.4% had a HR-positive disease and 43.2% had de novo metastases or occurring within 6 months after adjuvant treatment. DFI was >24 months in 47.9% of patients, and 78.5% received 1st-line trastuzumab combined with taxane-based chemotherapy (CT).

In 87 patients (25.6%) classified as LTR, median PFS was 56.2 months (CI95% 41.53-not reached) vs. 10.9 months in non-LTR (CI95% 9.46-11.8). Median OS in LTR is not reached versus 44.3 months in non-LTR (CI95% 37.9-49.1).

Median age, menopausal status, BMI, pathological characteristics and treatment for primary BC were similar in both groups. The following factors were predictive of LTR status by univariate analysis: number of metastatic sites (<3 versus ≥ 3, p=0.022); endocrine treatment for metastatic disease (p=0.002); absence of central nervous system metastases (p=0.035); and taxane-based 1st-line CT (p=0.032). In multivariate analysis, only number of metastatic sites and taxane-based 1st-line CT were predictive of LTR status.

In contrast, age, DFI, visceral disease, and adjuvant trastuzumab-based treatment did not influence outcome. Although not statistically significant, HR+ status was more frequent in LTR versus non-LTR patients (66.7% versus 56.9%).

Conclusions
With a median follow-up of 42.5 months, we found that more than 95% of LTR to 1st-line trastuzumab were still alive and could achieve a median PFS longer than 4.6 years. Although some clinical factors are clearly associated with better outcome, further investigations are needed to identify the mechanisms of resistance or sensitivity to trastuzumab.
Title: The synergistic antitumor activity of entinostat (MS-275) in combination with palbociclib (PD 0332991) in estrogen receptor-positive and triple-negative breast cancer

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Body: BACKGROUND: CDK4/6 regulates the G1-S phase transition by phosphorylating the retinoblastoma protein (Rb). Given their potent clinical efficacy, CDK4/6 inhibitors used in combination with hormone receptor (HR) blockade (with an aromatase inhibitor or fulvestrant) are emerging as the standard of care for patients with metastatic HR-positive breast cancers. The CDK4/6 inhibitors palbociclib and ribociclib are FDA-approved for use in HR-positive breast cancer patients, and abemaciclib is currently in phase III trials. We observed that approximately 74% (25/34) of breast cancer cell lines had high phosphorylated Rb (phospho-Rb) expression levels and that triple-negative breast cancer (TNBC) cell lines often expressed phospho-Rb, suggesting that targeting phospho-Rb via CDK4/6 inhibition may be effective against TNBC. The histone deacetylase (HDAC) inhibitors increase p21^{Cip1} levels, promoting proteasomal degradation of cyclin B1 and resulting in G2/M arrest. Entinostat is an oral, class 1, selective HDAC inhibitor currently in phase III testing in HR-positive breast cancer. Preclinical and clinical data demonstrate that entinostat, in combination with HR blockade, has anticancer activity. Our group recently reported that entinostat combined with other anticancer drugs induced apoptosis via induction of proapoptotic proteins such as Noxa and Bim in breast cancer cell lines. Based on these findings, we hypothesized that entinostat-induced apoptosis and palbociclib-induced cell cycle arrest synergize to produce enhanced antitumor effects in estrogen receptor (ER)-positive breast cancer and TNBC cell lines with high phospho-Rb expression levels.

METHODS: We assessed the combination antitumor effects and their mechanisms via CellTiter Blue and sulforhodamine B assays, flow cytometry, apoptosis (caspase 3/7) assays, anchorage-independent growth assays, Western blotting, reverse phase protein array (RPPA), and mammary fat pad xenograft mouse models.

RESULTS: RPPA data showed that ER-positive and TNBC cell lines more often expressed phospho-Rb than did other breast cancer cell subtypes (7/10 and 8/17 cell lines, respectively). We found that the combination of entinostat and palbociclib synergistically inhibited tumor cell proliferation (combinational index less than 1.0), reduced in vitro colony formation (P < 0.05), inhibited in vivo tumor growth in ER-positive MCF-7 breast cancer cells (P < 0.05), and inhibited tumor growth in TNBC xenograft mouse models (MDA-MB-231) more effectively than did either drug alone.

CONCLUSION: Taken together, our data provide evidence that combining entinostat with palbociclib enhances the antitumor effects of these drugs. Along with our continued effort to determine predictive biomarkers, our findings justify conducting a clinical trial of combination treatment with entinostat and palbociclib in patients with ER-positive breast cancer or TNBC.
Preclinical studies of RX-5902, a beta-catenin modulator in triple negative breast cancer

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RX-5902 (Supinoxin) is a novel anti-cancer compound that targets phosphorylated p68 RNA helicase, a member of the DEAD box family of helicases, affecting upstream and downstream molecules in the Wnt canonical pathway. As a single agent, RX-5902 exhibits strong growth inhibition in both in vitro and in vivo settings. Specifically, RX-5902 enhances survival and tumor growth inhibition in numerous xenograft models, including ovarian, renal and breast. We have previously shown RX-5902 inhibits cell growth in a dose-dependent fashion in the triple-negative breast cancer (TNBC) xenograft MDA-MB231. In the current study, we have expanded our investigation of the therapeutic potential of RX-5902 against TNBC using both in vitro and in vivo preclinical models.

RX-5902 was provided by Rexahn, Inc. (Rockville, MD). Cell proliferation was measured using the Cell-Titer Glo luminescence cell viability assay (Promega). Apoptosis was assessed using Incucyte Caspase 3/7 Green apoptosis assay (Essenbioscience). Immunoblots of MDA-MB-231 cell line were probed for β-catenin (Cell Signaling). Syngeneic 4T1 murine TNBC mice were obtained from Sippr-BK Laboratory Animal Co (Shanghai, China) and tumor volumes were measured twice a week. When the mean tumor volumes reached ~90 mm³, mice were randomized and treated with vehicle or RX-5902 PO daily alone or in combination with anti-CTLA4 or anti-PD-1 BIW for 3 weeks. Tumor growth inhibition (TGI) was calculated at Day 25.

A panel of 18 TNBC cell lines were treated with RX-5902 and effects on cell proliferation were measured by the Cell Titer-Glo assay. Using 100nM as a cutoff, 14 sensitive lines and 4 resistant lines were identified, with an average IC50 of 56 nM in the sensitive lines. Of these, we chose 2 sensitive lines (MDA-MB-231, HCC1806) and 2 resistant lines (MDA-MB-436 and CAL-120) and assessed induction of apoptosis by the Incucyte caspase activity assay. Robust induction of apoptosis was observed in both sensitive lines (N=3). These lines were then subjected to cell cycle analysis by flow cytometry, which revealed a pronounced G2/M cell cycle arrest and aneuploidy following exposure to RX-5902. Western blot analysis of the MDA-MB-231 cell line showed decreases in the Wnt pathway-related protein nuclear β-catenin in doses ranging from 20 nM to 200 nM. Finally, the therapeutic efficacy of RX-5902 was assessed as a single agent and in combination with two immune-oncology agents in the treatment of the TNBC 4T1 animal model. RX-5902 as a single agent showed dose dependency in the 4T1 model, and when given in combination with either anti-CTLA4 or anti-PD1 showed an additive effect (p<0.001). All the treatments were well-tolerated and no severe body weight loss was observed in this study.

RX-5902 showed efficacy against several in vitro and in vivo preclinical models of TNBC. RX-5902 resulted in G2/M arrest and induced apoptosis in sensitive TNBC cell lines and decreases in nuclear beta-catenin. In vivo, RX-5902 demonstrated additive anti-tumor effects when combined with either anti-CTLA4 or anti-PD1 immunotherapies. Together, these findings indicate that RX-5902 may have important clinical implications for the treatment of TNBC. A phase 2a clinical study in metastatic TNBC is ongoing.
Title: Efficacy, safety and tolerability of neratinib-based therapy in patients from Asia with metastatic HER2+ breast cancer and other solid tumors: A pooled analysis of 6 clinical trials

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Body: Background: HER2 overexpression/amplification occurs in ~15–20% of primary breast cancers (BC) in western populations, although the incidence of HER2+ BC in Asia may be higher (20–44% depending on the country). Neratinib is an irreversible tyrosine kinase inhibitor of HER1, 2 and 4, with demonstrated efficacy in trastuzumab-pretreated and trastuzumab-naïve HER2+ metastatic BC. To better understand the effects of neratinib in Asian patients (pts), we performed a pooled analysis of 6 phase I/II clinical trials in pts with metastatic HER2+ BC or other solid tumors.

Methods: Six prospective phase I/II or II clinical studies of neratinib, alone or in combination with other targeted or chemotherapeutic agents, in pts with metastatic HER2+ BC or other solid tumors were included. A pooled analysis of data from these trials was performed to compare efficacy and safety outcomes with neratinib-based therapy in pts from centers in Asian countries (China, Hong Kong, Japan, Korea, Malaysia, Singapore, and Taiwan) vs pts from other regions (Europe, North/South America, Australasia). Analyses were descriptive in nature. All trials were registered (Clinicaltrials.gov identifiers: NCT00445458; NCT00706030; NCT00398567; NCT00915018; NCT00741260; NCT00300781).

Results: A total of 966 pts were included (Asia, n=329; other regions, n=637). Most pts had HER2+ BC (96.8%); the remaining pts had other solid tumors (3.2%). Baseline characteristics were similar in pts from Asia vs other regions: median age, 52 vs 53 years; ECOG performance status 0/1, 98% vs 97%; hormone receptor-positive, 50% vs 48%. Neratinib was given as monotherapy (n=136) or in combination with paclitaxel (n=352), capecitabine (n=105), vinorelbine (n=91) or trastuzumab (n=45). Median duration of neratinib treatment in pts from Asia vs other regions was 338 vs 213 days; 47.3% vs 26.5% of pts received treatment for >1 year. Efficacy outcomes in pts with HER2+ BC are summarized in the table.

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Asia (n=239)a</th>
<th>Other regions (n=435)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORR, n (%)</td>
<td>171 (71.5)</td>
<td>243 (55.9)</td>
</tr>
<tr>
<td>CBR, n (%)</td>
<td>183 (76.6)</td>
<td>275 (63.2)</td>
</tr>
<tr>
<td>Median PFS (95% CI), weeks</td>
<td>56.1 (48.0-67.7)</td>
<td>39.3 (32.7-44.1)</td>
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</table>

CBR, clinical benefit rate; ORR, objective response rate; PFS, progression-free survival; a. Excluded phase I, non-BC and non-neratinib–treated pts

Incidence rates of grade 3/4 adverse events (Asia, 62.4% vs other regions, 66.0%) and grade 3/4 diarrhea were similar in both cohorts (25.6% vs 27.2%), but pts from Asia appeared to experience more grade 3/4 hematological events (neutropenia: 21.4% vs 9.8%; leukopenia: 13.0% vs 4.9%). Dose modifications were similar between cohorts, but Asian pts were less likely to withdraw from therapy (2.1% vs other regions, 4.7%).

Conclusions: Asian pts in the pooled metastatic trials appeared to have better ORR, CBR and PFS with neratinib-based therapy than pts from other regions. The safety and tolerability profile of neratinib was broadly similar between regions, except for a higher rate of grade 3/4 hematological events among Asian pts; however, Asian pts were less likely to withdraw from neratinib and stayed on treatment longer, a possible contributing factor to the better clinical outcomes observed in this cohort.
Subsequent treatment for postmenopausal women with hormone receptor-positive, HER2-negative advanced breast cancer who received ribociclib + letrozole vs placebo + letrozole in the phase III MONALEESA-2 study

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Background: In the Phase III MONALEESA-2 study (NCT01958021), ribociclib (RIB; cyclin-dependent kinase 4/6 inhibitor [CDK4/6i]) + letrozole (LET) significantly prolonged progression-free survival (PFS) vs placebo (PBO) + LET in postmenopausal women with hormone receptor-positive (HR+), human epidermal growth factor receptor 2-negative (HER2–) advanced breast cancer (ABC). The optimal treatment sequence following first-line CDK4/6i-based therapy is not yet known. Here we report the subsequent therapies received following discontinuation from MONALEESA-2.

Methods: The MONALEESA-2 study enrolled 668 patients (pts) with HR+, HER2– ABC. Pts were randomized 1:1 to receive RIB (600 mg/day; 3-weeks-on/1-week-off) + LET (2.5 mg/day; continuous) or PBO + LET. Following discontinuation of MONALEESA-2 study treatment, pts were followed for information regarding post-study treatment, including type and duration of therapy.

Results: At data cut-off (January 2, 2017), the median duration of follow-up was 26.4 months. Median PFS was 25.3 vs 16.0 months in the RIB + LET vs PBO + LET arms (hazard ratio=0.568; 95% confidence interval [CI]: 0.457–0.704; p=9.63x10⁻⁸). 203 (60.8%) vs 246 (73.7%) pts had discontinued RIB + LET vs PBO + LET. The median time to end of treatment was 20.3 months in the RIB + LET arm vs 13.7 months in the PBO + LET arm. First subsequent antineoplastic treatment was reported for 172/203 (84.7%) vs 212/246 (86.2%) pts who received RIB + LET vs PBO + LET; second subsequent therapy was reported for 45/203 (22.2%) vs 68/246 (27.6%) pts. The median time to first subsequent therapy (from randomization to the first post-study dose of therapy) was 24.2 (95% CI: 20.9–27.6) vs 16.7 (95% CI: 14.8–19.3) months in pts who received RIB + LET vs PBO + LET; median time to initiation of second subsequent therapy was not reached in either arm. The most common type of first subsequent therapy was single-agent hormonal therapy in 90 (44.3%) vs 87 (35.4%) pts who discontinued RIB + LET vs PBO + LET; chemotherapy was the most common second subsequent therapy in 20 (9.9%) vs 36 (14.6%) pts. Chemotherapy alone was the first subsequent treatment after MONALEESA-2 discontinuation in 32 (15.8%) vs 55 (22.4%) pts treated with RIB + LET vs PBO + LET.

Conclusions: RIB + LET significantly prolongs PFS and delays the start of subsequent lines of therapy vs PBO + LET in pts with HR+, HER2– ABC. The most common first subsequent therapy following discontinuation of RIB + LET or PBO + LET was single-agent hormonal therapy, and fewer pts treated with RIB + LET received subsequent chemotherapy compared with those who received PBO + LET.
Title: Suppression of breast carcinogenesis and metastasis by targeting glucose metabolism with HJC0152

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Body: Lack of targeted strategies for preventing and treating estrogen receptor (ER)-negative breast cancer (ENBC) is an unmet clinical challenge. ENBCs including triple-negative BCs (TNBC) constitute 30-40% of BC cases and are prone to develop remote metastasis and local recurrence, resulting in the majority of deaths in BC patients. Dysregulated glucose and energy metabolism is critically involved in the development and progression of various cancers via promoting aberrant cell growth, malignant transformation and metastasis. Nevertheless, the potential role of glucose/energy metabolism in ENBC carcinogenesis has sparsely been explored, thus representing a key knowledge gap and a potential avenue for effective targeted therapies. Despite a substantial amount of effort has been made towards anticancer metabolic and biogenetic medications, none has progressed into clinical use, due to their limited potency, specificity or drug properties such as toxicity and poor bioavailability. We recently developed HJC0152, a novel small molecule anticancer agent, using structure- and fragment-based drug design strategies and molecular modeling techniques in our initial attempt to develop non-peptide STAT3 inhibitors for anticancer use. HJC0152 significantly inhibits proliferation of BC cells, induces apoptosis, reduces ER-negative mammary tumor development, suppresses ENBC xenograft tumor growth, and blocks lung metastasis in vivo. Intriguingly, HJC0152 differentially modulates expression of glycolytic enzymes including HK1, PFK-L, PFKFB2, ENO2, PDH, PDK1, PGAM1 and ALDOA in a time-dependent manner. HJC0152 also regulates the transcription of genes involved in glucose and mitochondrial energy metabolism, including the subunits of mitochondrial respiratory chain complexes. Functional assessments further demonstrate that HJC0152 significantly modulates respiratory chain function. Via in silico and Unique Polymer Technology (UPT) strategy, we identified a number of putative HJC0152-interacting targets for validation studies. Our findings suggest that HJC0152 is capable of reprogramming/restoring the dysregulated glucose metabolism by inducing specific glycolytic enzyme expression and mitochondrial respiratory chain function, likely via targeting upstream key signal molecule(s) that regulates glucose and energy metabolism, thereby suppressing breast cancer development and progression to metastasis. This work was supported by Grants P50 CA097007, and P30DA028821 (JZ) from the NIH, CPRIT (JZ), John Sealy Memorial Endowment Fund (JZ), DFI Grants from MD Anderson Cancer Center (QS), Holden Family Research Grant in BC Prevention (QS), and NCI PREVENT Program HHSN26100002 (QS).
Title: Integrating comprehensive genomic profiling with treatment decisions – Experience gained while treating 139 advanced breast carcinomas

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Body: Background: Comprehensive Genomic Profiling (CGP) using next-generation sequencing (NGS) technology can provide insight into potentially clinically relevant genomic alterations (CRGA) within a patient's breast cancer. For example, HER2 amplification status and targetable short variants (SV), acquired ESR1 or BRCA1/2 resistance mutations, and the presence of targetable alterations in the PI3K kinase. We retrospectively reviewed CGP results and subsequent outcomes at one cancer center to illustrate the experience of using molecular subtyping to inform treatment decisions.

Methods: DNA extracted from FFPE tumor tissue or blood samples obtained during routine clinical care for patients (n=139) with predominantly relapsed, refractory or metastatic breast cancer was analyzed by hybrid-capture, NGS for all classes of GA: 1. base substitutions, 2. insertion and deletions, 3. rearrangements, and 4. copy number changes. Treatment decisions based on comprehensive genomic profiles were captured retrospectively. Tumor mutational burden (TMB), scored as mutations (mut)/Mb, was calculated on 0.8-1.2 Mb of sequenced DNA. Alterations affecting the ERBB family included amplification of or oncogenic mutations in ERBB2 (HER2), ERBB3, and EGFR.

Results: From Jan 2013 to May 2017, FFPE tissue samples for 136 patients with advanced breast cancer were analyzed by CGP and 3 additional patients had circulating tumor DNA analyzed for alterations; 11 patients received profiling on multiple biopsies. Tumors analyzed were carcinomas (Ca) NOS (n=84), invasive ductal Ca (n=46), invasive lobular Ca (n=7), a neuroendocrine Ca, and a phyllodes tumor. In total, 118/139 (84.9%) samples harbored CRGA in a targetable pathway: PI3K/MTOR (n=67; 48.2%), CDK cell-cycle (n=40; 28.8%), ERBB family (n=24; 17.3%), FGFR (n=24; 17.3%), ESR1 (n=16; 11.5%), homologous repair (HRD)( n=14; 10.1%), and RAS/RAF/MEK (n=11; 7.9%). Targetable alterations in other cancer-related kinases were found in 10 (7.2%) samples and 10 (7.2%) samples were TMB high (≥20 mut/Mb) or had CD274 (PD-L1) amplification. There were 3 patients (2.1%) with HER2 short variants detected in the absence of ERBB2 amplification; these patients may respond to HER2-targeted therapies but would be HER2-negative by IHC. Many samples had alterations in ≥1 pathway, and overlap is particularly high for the CDK and FGFR pathways (12 samples). Alterations in pathways targeted by MTOR inhibitors, HER2-targeted therapies, or the CDK inhibitors were found in 93/136 (66.9%) tumors. Evaluation of outcomes for these 139 patients is ongoing and will be presented.

Conclusions: Genomic profiling of breast carcinomas, using either tissue or liquid biopsies, provides potentially actionable information to guide treatment decisions. Overall, 84.9% of patient samples harbored oncogenic alterations in a targetable pathway, with two-thirds of tumors having alterations in pathways targeted by therapies with FDA approval for breast cancer and 7.2% of patients having high levels of TMB or amplification of PD-L1, suggesting that checkpoint inhibitors may be relevant options.
2017 San Antonio Breast Cancer Symposium

Publication Number: P5-21-21

**Title:** Palbociclib exposure-response analyses in the treatment of hormone-receptor positive (HR+), human epidermal growth factor receptor 2 negative (HER2–) advanced breast cancer (ABC)

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¹Pfizer, San Diego, CA; ²Institut Curie Paris and Centre Eugène Marquis, Rennes, France; ³Geffen School of Medicine at UCLA, Santa Monica, CA and ⁴Clinical Pharmacology, Pfizer, San Diego, CA.

**Body:** Palbociclib (PAL) is approved for the treatment of HR+, HER2- ABC in combination with an aromatase inhibitor as initial endocrine based therapy in postmenopausal women or with fulvestrant in women with disease progression following endocrine therapy based on major progression-free survival (PFS) improvement in phase III studies. An exposure response analysis was conducted to evaluate how the changes in PAL exposure (PAL-E), e.g. due to the dose modifications, would affect the PFS. Data from PALOMA 2, a phase 3 study comparing the efficacy and safety of letrozole plus PAL (PAL+L) to letrozole plus placebo (L) in ABC patients (PTs), were used. The apparent PAL clearance (CL/F) for each PT was obtained from a population pharmacokinetic analysis. The individual PAL-E over the entire treatment (C_{avg}) was calculated using average daily dose intensity (ADI) and CL/F. PTs in the PAL+L group were divided into 4 quantiles (Q1, Q2, Q3, and Q4) based on their C_{avg}, and the median PFS in each quantile was compared to that of L using Kaplan-Meier plot. To account for the changes in PAL-E over time due to dose modifications during treatment, a time-varying PAL-E (C_{avgt}) was calculated using ADI and CL/F at each PFS time. The relationship between PFS and C_{avgt} was evaluated using Cox proportional hazard model. Univariate analyses were first conducted to identify any significant prognostic factors and the effect of C_{avgt} on PFS. Multivariate analysis was conducted to evaluate relationship between PFS and C_{avgt} when all significant prognostic factors identified from the univariate analysis were included in the model to account for their potential confounding effects.

The median PFS were 14.5, 24.9, 27.7, 25.7, and 24.0 months for patients in the L, Q1, Q2, Q3, and Q4, respectively, indicating similar PFS across all exposure quartiles. The identified significant prognostic factors from univariate analysis were Ki67 score, age, baseline AST, baseline tumor size, baseline alkaline phosphatase, and baseline albumin levels. The estimated coefficients for C_{avgt} were -0.00377 and -0.00224 in univariate and multivariate analysis, respectively. The effect of PAL-E on PFS was not statistically significant (p <0.05).

PFS was not found to be associated with C_{avgt}, suggesting that PTs with different PAL-E in P+L benefited similarly and the changes in PAL-E resulted from dose modifications in the trial would not significantly affect the efficacy. However, the impacts on efficacy are unknown for any other unstudied dose modifications algorithm.
Title: Palbociclib in highly pretreated metastatic ER-positive HER-2 negative breast cancer

Griet Hoste, Kevin Punie, Hans Wildiers, Patrick Neven, Patrick Berteloot, Els Van Nieuwenhuysen, Sileny Han, Nicole Concin, Rawand Salihi, Inge Lefever and Ignace Vergote. UZ Leuven, Leuven, Vlaams-Brabant, Belgium.

Body: Palbociclib, a first-in-class CDK 4/6 inhibitor, in combination with letrozole or fulvestrant is now licensed for the first or second line treatment of postmenopausal women with hormone-sensitive HER-2 negative metastatic breast cancer (MBC), but its activity in later lines is unknown. In Belgium, a compassionate Use Program (CUP) was temporarily established to provide palbociclib after at least 4 lines of systemic treatment for MBC.

The UZ Leuven Multidisciplinary Breast Center included 82 patients in this CUP from September 28th 2015 to March 14th 2017. This analysis describes retrospectively collected efficacy (Revised RECIST guideline, version 1.1) and safety data from 68 patients with at least 6 months follow-up at the data cut-off point. The primary endpoint was clinical benefit, defined as being on treatment for at least 6 months (CR + PR + SD). Most of the patients (89.7%) used palbociclib in combination with letrozole. Other combinations are with tamoxifen (2.9%), fulvestrant (2.9%), exemestane (1.5%), anastrazole (1.5%) and megestrol (1.5%). At the data cut-off point, 18 patients are still on-treatment with palbociclib. The average duration of treatment is 5.7 months [range 2m- 17m]. The mean age of the patients was 66.3 years [range 34.8y – 85.9y] at the time of inclusion. Patients had had an average of 5.7 lines of systemic therapy [range 4 – 11 lines] before starting palbociclib, which was in 61.8% at least one line of chemotherapy. In this highly pretreated setting, 29 patients experienced stable disease lasting ≥ 6 months, resulting in an overall clinical benefit rate of 42.6%. 19.1% (13/68) showed stable disease for ≥ 9 months and 8% for ≥ 12 months. The subjective tolerance of the combination treatment was good, with 38% (26/68) of the patients discontinuing or delaying treatment following adverse events which were in the vast majority hematologic but asymptomatic. No factors predicting clinical benefit could be identified: use of chemotherapy before starting Palbociclib (p = 0.4644), age (p = 0.7029), time between primary breast cancer diagnosis and starting palbociclib (p = 0.1919) or time between first metastasis and starting palbociclib (p = 0.1108) and bone-only disease (p = 1,0000) were not significantly associated with clinical benefit at 6 months.

These data not only support the findings of the PALOMA studies, but also show unexpectedly high clinical benefit and safety of palbociclib in heavily pretreated endocrine-resistant hormone receptor positive HER-2 negative advanced breast cancer. An update of these data will be presented.
**Title:** Evaluation of the drug interaction potential of palbociclib and exemestane – Results from the PEARL pharmacokinetic sub-Study

Miguel Martín1,2,3, Justin Hoffman4, Manuel Ruiz-Borrego5,2, Montserrat Muñoz5,2, Lourdes Calvo7,2, Penelope Crownover12, J Angel García-Sáenz8,2, Emilio Alba8,2,3, Diane Wang4, Christiane Thallinger10, Agostina Stradella11,2, Álvaro Montaño5,2, Bárbara Adamo6,2, Silvia Antolín7,2, Fernando Moreno-Antón8,2, Catalina Falo11,2, Victoria Ruiz2, Nuria Martín2, Rosalia Caballero2, Eva Carrasco2 and Miguel Gil-Gil11,2. 1Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain; 2GEICAM Spanish Breast Cancer Group, Madrid, Spain; 3Centro de Investigación Biomédica en Red de Oncología, CIBERONC-ISICII, Madrid, Spain; 4Pfizer, Inc, CA; 5Hospital Universitario Virgen del Rocío, Madrid, Spain; 6Hospital Universitari Clinic de Barcelona, Barcelona, Spain; 7Complejo Hospitalario Universitario A Coruña, A Coruna, Spain; 8Hospital Universitario San Carlos, Madrid, Spain; 9Hospital Universitario Regional y Virgen de la Victoria. IBIMA, Malaga, Spain; 10Univ. Klinik für Innere Medizin I, Währinger Gürtel, Vienna, Austria; 11Institut Catalá d’Oncologia (ICO), L’Hospitalet, Barcelona, Spain and 12Pfizer, Inc., CT.

**Body:** Background: Palbociclib (PAL) is an oral cyclin-dependent kinase (CDK) 4/6 inhibitor that is under investigation in multiple oncologic clinical trials and is currently approved for use in combination with aromatase inhibitors (AIs) or fulvestrant (FUL) in patients with hormone receptor positive (HR+), human epidermal growth factor receptor 2 negative (HER2–) advanced breast cancer (BC).

The PEARL Study is an ongoing international, open label, controlled, randomized Phase 3 study comparing the efficacy and safety of PAL in combination with endocrine therapy (exemestane [EXE] or FUL) versus capecitabine in postmenopausal women with HR+/ HER2– metastatic BC whose disease progressed on AIs. A secondary objective of the study was to evaluate the pharmacokinetics (PK) of PAL (125mg QD, 3 weeks on/1 week off) and EXE (25mg QD, continuously) when coadministered. This is the first study to investigate the drug-drug interaction (DDI) potential of the combination of PAL and the AI EXE.

**Methods:** Patients (pts) randomized to the PAL+EXE arm of the PEARL Study in seven selected sites had the option of participating in the PK sub-study. Those who enrolled in the PK sub-study received EXE alone in a 7-day lead-in period immediately prior to Cycle 1 Day 1, when both drugs were coadministered on their standard dosing regimens. Sub-study pts were to have 2 pre-dose plasma PK samples drawn at steady-state (ss) during the lead-in period (“EXE Alone”) for EXE determination, and 2 ss PK samples drawn for EXE and PAL determination (2 per analyte) during coadministration (“PAL+EXE”). Plasma concentrations of PAL and EXE were measured using validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods. The withinpatient mean concentration of the PK samples which met ss acceptance criteria (WPM-Ctrough) for each analyte were generated for each treatment period as the input for DDI analyses.

To assess the effect of coadministration of PAL on EXE PK, the WPM-Ctrough of EXE was compared within patients between the "PAL+EXE" (Test) and “EXE Alone” (Reference) treatment periods using a one-way analysis of variance (ANOVA) model with treatment as a fixed effect and patient as a random effect. To assess the effect of coadministration of EXE on PAL PK, the WPM-Ctrough of PAL was compared between the “PAL+EXE” period (Test) and historical data (Reference) using an ANOVA model. Analysis of covariance (ANCOVA) models were used to assess the impact of demographic differences between analysis populations in covariates known to impact PAL PK on the ANOVA model conclusions.

**Results:** A total of 26 pts randomized to the PAL+EXE arm were enrolled in the PK sub-study and had PK samples analysed, of which 23 meet ss acceptance criteria. The ratio of the adjusted geometric means for EXE WPM-Ctrough was 106.9% (90%CI: 82.4-138.8), when EXE was administered with PAL, compared with its administration alone. Likewise, the models to assess potential for EXE to perpetrate DDI on PAL PK showed ratios of adjusted geometric means of 102.4% (90%CI: 82.0-127.9) and 111.6% (90%CI: 90.3137.8), when adjusted for covariates.

**Conclusion:** The PK data indicate a lack of a clinically meaningful DDI between PAL and EXE when the 2 drugs are coadministered.

**Sponsor:** GEICAM
Body: Background: The objective of this study was to evaluate treatment patterns and clinical outcomes among patients who received palbociclib in combination with letrozole (P+L) for the treatment of HR+/HER2–advanced breast cancer (ABC) as part of an Expanded Access Program (EAP) in the United States.

Methods: Data were obtained by a retrospective chart review of patients previously enrolled in the EAP. Complete data from time of initial diagnosis of ABC until the date of chart abstraction (end of follow-up), including the post-EAP period, were obtained. Clinical outcomes assessed included clinical benefit rate (CBR), defined as complete response, partial response, or stable disease for ≥24 weeks from P + L initiation, progression free survival (PFS) and overall survival (OS). Survival outcomes were assessed using the Kaplan-Meier statistical analysis.

Results: Data from 126 patients were included in this analysis. Median age was 62.5 years at EAP enrollment, and a majority of patients were Caucasian (83%). Approximately 25% of patients had de novo metastatic disease. A majority of patients had a performance status of ECOG 0 (56%) or 1 (37%) at EAP enrollment. Visceral disease was present in 71% of patients and 16% had bone-only disease. The majority of patients in this cohort from the EAP were heavily pre-treated, having had up to 5 prior lines of therapy in the metastatic setting prior to initiating P + L therapy; nearly 59% received 3+ prior lines before initiating P + L. Only 11% of patients received P + L as their initial regimen for MBC. At the time of the last available record, 12 patients were still on P + L therapy, an average of 21 months after the start of the EAP program. Nearly 80% of patients had prior AI exposure and 69% had prior chemotherapy. CBR was 33% for the overall sample of patients treated with P + L and 22% in those with 3+ prior lines of treatment. Patients with prior AI exposure in the ABC setting (n=100) had a CBR of 27% while those without prior AI exposure had CBR of 58%. Patients with prior chemotherapy (n=87) had a CBR of 28% and those without prior chemotherapy had CBR of 46%. For the entire cohort, 6- and 12-month PFS rates were 40% and 25% respectively; 12- and 24-month OS rates were 66% and 44%, respectively. Patients receiving 3+ lines of prior therapy had 6- and 12-month PFS rates of 28% and 19%, respectively, and 12- and 24-month OS rates of 59% and 34% respectively.

Conclusions: Our results suggest that the majority of patients enrolled in the EAP program derived benefit from receiving treatment with P + L despite multiple prior lines of treatment and prior endocrine-based therapy, including prior AI. These findings further demonstrate the benefit of treatment with palbociclib combination therapy in HR+/HER2– MBC.
Title: Efficacy and safety of palbociclib (PAL) + letrozole (LET) as first-line therapy in estrogen receptor–positive (ER+)/human epidermal growth factor receptor 2–negative (HER2−) advanced breast cancer (ABC): Findings by geographic region from PALOMA-2

Karen A Gelmon1, Aurelio Castrellon2, Anil A Joy3, Janice M Walshe4, Johannes Ettl5, Hirofumi Mukai6, In Hae Park7, Dongrui R Lu8, Ave Mori9, Eustratios Bananis10, Véronique Diéras11 and Richard S Finn12. 1British Columbia Cancer Agency, Vancouver, BC, Canada; 2Memorial Cancer Institute, Breast Cancer Center, Pembroke Pines, FL; 3Cross Cancer Institute, University of Alberta, Edmonton, AB, Canada; 4Cancer Trials Ireland, Dublin, Ireland; 5Frauenklinik und Poliklinik Klinikum rechts der Isar, Technische Universität München, München, Germany; 6National Cancer Center Hospital East Tokyo, Tokyo, Japan; 7National Cancer Center, Goyangsi, Korea; 8Pfizer Inc, La Jolla, CA; 9Pfizer S.r.l., Milan, Italy; 10Pfizer Inc, New York, NY; 11Institut Curie, Paris, France and 12David Geffen School of Medicine at UCLA, Santa Monica, CA.

Body: BACKGROUND: Previous findings from the PALOMA-2 study (N=666) demonstrated the efficacy and safety of PAL+LET as first-line ABC therapy versus placebo (PBO)+LET (Finn et al, NEJM. 2016). This analysis evaluated the efficacy and safety of PAL+LET by geographic region (North America [NA], Europe [EU], and Asia Pacific [AP]; data cutoff: Feb 26, 2016).

METHODS: Women with ER+/HER2– ABC who had not received prior systemic treatment in the advanced setting were randomized 2:1 to PAL (125 mg/d oral [3 wks on, 1 wk off])+LET (2.5 mg once daily) or PBO+LET.

RESULTS: This analysis included 267 patients from NA, 307 from EU, and 92 from AP. At baseline, demographics and disease characteristics generally were similar between regions. In the overall population (Table 1), PAL+LET demonstrated improvements versus PBO+LET in progression-free survival (PFS), objective response rate (ORR), and clinical benefit response rate (CBR). Similarly, PFS was longer and ORR and CBR were higher with PAL+LET versus PBO+LET in NA, EU, and AP subgroups (Table 1). All-grade treatment-emergent adverse events (AEs) (PAL+LET/PBO+LET) occurred in 99%/99% of patients in NA, 98%/92% in EU, and 100%/96% in AP. In the PAL+LET arm, neutropenia (all-grade/grade ≥3) was the most common AE in all regions. The incidence of neutropenia was numerically higher in AP (91%/84%) compared with NA (73%/65%) and EU (81%/62%). Grade 3 or 4 febrile neutropenia occurred in 4 (2%) NA patients, 4 (2%) EU patients, and no AP patients in the PAL+LET arm and in no patients in any of the regions in the PBO+LET arm.

CONCLUSIONS: PAL+LET showed improvement versus PBO+LET in PFS, ORR, and CBR in patients with ER+/HER2- ABC in NA, EU, and AP, with comparable magnitude of benefit between regions. With PAL+LET, neutropenia was the most commonly reported AE in all regions, with a numerically higher incidence reported in AP versus NA or EU; the safety profile was similar to previously reported results in the overall population.

Funding: Pfizer (NCT01740427)

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Table 1. PFS, ORR, and CBR

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<tr>
<th></th>
<th>Median PFS (95% CI), mo</th>
<th>PFS HR (95% Cl)</th>
<th>ORR,* % (95% CI)</th>
<th>CBR,* % (95% CI)</th>
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<tr>
<td><strong>Overall Population</strong></td>
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<td>PAL+LET</td>
<td>24.8 (22.1-NE)</td>
<td>0.58 (0.46-0.72); P&lt;0.001</td>
<td>55.3 (49.9-60.7)</td>
<td>84.3 (80.0-88.0)</td>
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<td>PBO+LET</td>
<td>14.5 (12.9-17.1)</td>
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<td>44.4 (36.9-52.2)</td>
<td>70.8 (63.3-77.5)</td>
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<td>PAL+LET</td>
<td>24.2 (17.5-NE)</td>
<td>0.61 (0.43-0.85)</td>
<td>54.3 (45.3-63.2)</td>
<td>80.3 (72.3-86.8)</td>
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<td>PBO+LET</td>
<td>13.8 (10.3-22.1)</td>
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<td>50.6 (39.1-62.1)</td>
<td>67.1 (55.6-77.3)</td>
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<td><strong>EU</strong></td>
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<tr>
<td>PAL+LET</td>
<td>24.8 (22.1-NE)</td>
<td>0.57 (0.41-0.80)</td>
<td>55.6 (47.6-63.5)</td>
<td>87.5 (81.4-92.2)</td>
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<td>PBO+LET</td>
<td>16.5 (11.3-19.6)</td>
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<td>38.2 (26.7-50.8)</td>
<td>73.5 (61.4-83.5)</td>
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<td>AP</td>
<td>PAL+LET</td>
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<td>22.2 (19.4-25.7)</td>
<td>13.9 (7.4-22.0)</td>
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<td></td>
<td>0.49 (0.27-0.87)</td>
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<td>56.9 (42.2-70.7)</td>
<td>41.7 (22.1-63.4)</td>
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<td>84.3 (71.4-93.0)</td>
<td>75.0 (53.3-90.2)</td>
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HR=hazard ratio; NE=not estimable; OR=objective response.*Confirmed OR in patients with measurable disease.
Title: T-DM1 activity in metastatic HER2-positive breast cancer patients who have received prior trastuzumab and pertuzumab: NSABP B-005

Shruti R Tiwari¹, Tamara Sussman¹, Karthik Kota², Halle CF Moore¹, Alberto J Montero¹, George T Budd¹, Shannon Puhalha² and Jame Abraham¹. ¹Cleveland Clinic Taussig Cancer Institute, Cleveland, OH and ²University of Pittsburgh, Pittsburgh, PA.

Body: Background:
The pivotal phase III EMILIA trial reported a progression free survival (PFS) rate of 9.6 months and an objective response rate of 43% with T-DM1 in patients with HER2-positive metastatic breast cancer previously treated with trastuzumab and a taxane. However, there is very limited data on the efficacy of T-DM1 in patients who have received prior pertuzumab either neoadjuvantly or as first line therapy in the metastatic setting. The primary goal of this study was to assess the clinical efficacy (tumor response rates and median duration on therapy) of T-DM1 in patients previously treated with pertuzumab and trastuzumab.

Methods:
After IRB approval, a cancer data registry and electronic pharmacy database were utilized to identify breast cancer patients receiving treatment with T-DM1 at Cleveland Clinic and University of Pittsburgh. Patients that received trastuzumab and pertuzumab, in either the neoadjuvant or metastatic setting, with baseline and follow up imaging available for review were identified. Patient charts were reviewed to collect accurate information about the treatment sequencing and outcomes. RECIST version 1.1 was utilized for tumor assessment and patients with measurable disease and non measurable disease were included in the study.

Results:
We identified a total of 23 patients with a median age of 55 years that met the inclusion criteria. 69% percent of patients received T-DM1 as first line or second line therapy and 31% received it as third line or later. All patients had at least 1 measurable lesion. Best overall response showed rates of complete response, partial response and stable disease of 17%, 26% and 22% respectively. 35% patients progressed on first assessment after start of treatment. The median duration on therapy was 5.3 months (range 3 weeks to 33 months) with 43% of patients receiving T-DM1 for greater than 6 months.

Conclusion:
Our results were comparable to those reported by EMILIA trial. T-DM1 has reasonable clinical efficacy in patients who have received prior treatment with pertuzumab and trastuzumab with an overall response rate of 43% and median duration on therapy of 5.3 months.
2017 San Antonio Breast Cancer Symposium

**Publication Number:** P5-21-27

**Title:** Efficacy and safety of ribociclib plus letrozole in US patients enrolled in the MONALEESA-2 study

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**Body: Background:** Endocrine-based therapy is the standard of care for patients with hormone receptor–positive (HR+) human epidermal growth factor receptor 2–negative (HER2−) advanced breast cancer (ABC), in whom delaying disease progression is the primary goal. In the randomized Phase 3 MONALEESA-2 study (ClinicalTrials.gov identifier, NCT01958021), first-line ribociclib (a selective inhibitor of cyclin-dependent kinases 4 and 6) + letrozole significantly prolonged progression-free survival (PFS) compared with placebo + letrozole in postmenopausal women with HR+, HER2− ABC (hazard ratio, 0.556; 95% confidence interval [CI], 0.429–0.720) at the interim analysis cutoff date. Here, we present safety and efficacy data from US patients who were enrolled in the MONALEESA-2 study.

**Methods:** Postmenopausal women (N=668) with HR+, HER2− ABC who did not receive prior systemic treatment for ABC and had an Eastern Cooperative Oncology Group performance status score of ≤1, adequate bone marrow and organ function, and no history of active cardiac dysfunction were randomized 1:1 to receive either ribociclib (600 mg/d, 3 weeks on/1 week off) + letrozole (2.5 mg/d, continuous) or placebo + letrozole. The primary end point was locally assessed PFS. The data cutoff for this analysis was January 29, 2016.

**Results:** Baseline demographics, patient disposition, and prior therapy were well balanced across treatment groups in the US subset (N=213; ribociclib group, n=100; placebo group, n=113). Median treatment duration was 16.3 months. In US patients, median PFS was significantly prolonged in the ribociclib group (median PFS, not reached [NR]; 95% CI, 17.1 months to NR) versus the placebo group (median PFS, 14.4 months [95% CI, 11.1–18.4 months]; hazard ratio, 0.451 [95% CI, 0.281–0.724]; P=0.000363), with a median follow-up of 11.0 months. In a subsequent preplanned analysis of overall survival, median PFS was 27.6 months in the ribociclib group versus 15.0 months in the placebo group (hazard ratio, 0.527; 95% CI, 0.351–0.793). The overall response rate (ORR) was significantly greater in the ribociclib group (ORR, 38.0%; 95% CI, 28.5–47.5%) versus the placebo group (ORR, 26.5%; 95% CI, 18.4–34.7%; P=0.040). A trend toward benefit was observed with ribociclib in all subgroups (including age, disease burden, prior therapy, and biomarker status), which was consistent with the overall population. No on-treatment deaths occurred. The most common all-grade adverse events, irrespective of causality, were neutropenia (ribociclib, 69.0%; placebo, 3.7%), nausea (ribociclib, 65.0%; placebo, 39.4%), fatigue (ribociclib, 56.0%; placebo, 46.8%), and diarrhea (ribociclib, 41.0%; placebo, 32.1%). The most common grade 3 (≥25% in either group) and grade 4 (≥10% in either group) AE was neutropenia (grade 3: ribociclib, 39.0%; placebo, 0%; grade 4: ribociclib, 14.0%; placebo, 0%). Two patients (2.0%) in the ribociclib group and none in the placebo group experienced febrile neutropenia.

**Conclusions:** The US patients in the MONALEESA-2 study derived significant PFS benefit from ribociclib + letrozole compared with placebo + letrozole, with a 55% reduction in the relative risk of disease progression. The safety profile was consistent with and comparable to the global study population.
Body: Background: First-line ribociclib (RIB; a selective cyclin-dependent kinase 4/6 inhibitor) + letrozole (LET) significantly prolonged progression-free survival vs placebo + LET in patients (pts) with hormone receptor-positive (HR+), human epidermal growth factor receptor 2-negative (HER2–) advanced breast cancer (ABC). The Phase Ib MONALEESASIA dose-escalation/-expansion study (NCT02333370) is investigating RIB + LET in Asian pts with HR+, HER2– ABC; here we report results in Asian non-Japanese pts.

Methods: Asian, non-Japanese postmenopausal women with HR+, HER2– ABC, with no prior systemic treatment for ABC, received RIB (starting dose 400 mg/day; 3-weeks-on/1-week-off; option to escalate or de-escalate) + LET (2.5 mg/day; continuous) until disease progression or discontinuation for other reasons. Dose escalation was guided by a Bayesian Logistic Regression Model (BLRM) with overdose control. The primary objective of the dose-escalation part was to estimate the maximum tolerated dose (MTD) and/or recommended Phase II dose (RP2D), based on the BLRM, safety data, and pharmacokinetic analyses; secondary/exploratory objectives included safety and tolerability, and antitumor activity.

Results: At data cut-off (January 16, 2017), 26 pts were enrolled; 13 in the dose escalation (RIB 400 mg: n=6; RIB 600 mg: n=7) and 13 in the 600 mg dose expansion. Treatment was ongoing in 18 pts (69%; 400 mg: n=3; 600 mg: n=15); the most common reason for discontinuation was disease progression (400 mg: n=3; 600 mg: n=4). One dose-limiting toxicity occurred at 600 mg (Grade 3 increased alanine transaminase [ALT]). The MTD/RP2D was declared as 600 mg/day RIB (3-weeks-on/1-week-off) + 2.5 mg/day LET (continuous). The most common all-grade, all-cause adverse events (AEs; ≥35% in either cohort; 400 mg vs 600 mg) were neutropenia (n=1 [17%] vs n=8 [40%]), increased ALT (n=1 [17%] vs n=7 [35%]), decreased neutrophil count (n=3 [50%] vs n=7 [35%]), and alopecia (n=3 [50%] vs n=2 [10%]). All-cause Grade 3/4 AEs occurred in 4 pts (67%) at 400 mg and 18 pts (90%) at 600 mg; the most common (≥25% in either cohort; 400 mg vs 600 mg) were decreased neutrophil count (n=3 [50%] vs n=7 [35%]) and neutropenia (n=1 [17%] vs n=6 [30%]). At least one RIB dose reduction due to AEs occurred in 2 pts at 400 mg and 10 pts at 600 mg. At least one RIB dose interruption due to AEs occurred in 4 pts at 400 mg and 13 pts at 600 mg. Post-baseline QTcF >480 ms occurred in 0 pts at 400 mg and 4 pts (20%) at 600 mg. No pt had a post-baseline QTcF >500 ms. The overall response rate was 50% (n=3) at 400 mg and 35% (n=7) at 600 mg (all partial responses). Stable disease was reported in 1 pt (17%) at 400 mg and 8 pts (40%) at 600 mg.

Conclusions: RIB + LET demonstrated a manageable safety profile with preliminary signs of antitumor activity in Asian non-Japanese pts with HR+, HER2– ABC; the safety profile was broadly consistent with that seen in non-Asian pts. The MTD/RP2D was declared as 600 mg/day RIB (3-weeks-on/1-week-off) + 2.5 mg/day LET (continuous). Further safety and efficacy data for RIB + LET will be reported in this pt population after the dose expansion is completed.
2017 San Antonio Breast Cancer Symposium

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Title: Moving from the CLEOPATRA study to real life: Preliminary results from the G.O.N.O. SUPER trial

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Body: Background: Around 20% of breast cancers (BC) are HER-2+. Trastuzumab (T) has dramatically changed the outcome of HER-2+ BC patients (pts), both in early and in advanced settings. The combination of Pertuzumab (P) plus T and docetaxel, used as first-line treatment for HER-2+ metastatic BC (MBC) in the phase III CLEOPATRA study, significantly prolonged progression free survival (PFS) and overall survival (OS).

In order to verify the results of the Cleopatra trial in unselected pts, we performed a multicenter, retrospective-prospective, observational study, in HER-2+ MBC pts candidate to receive first line therapy with P plus T plus taxanes.

Methods: We analyze the outcome of all HER-2+ MBC pts treated with P+T and taxanes, as first line therapy since the availability of P in Italy (2013), at 13 general and university hospitals.

Results: Until June 6th data from 217 HER-2+ MBC pts were recorded. Main pts’ characteristics were: median age 54 y (28-80), median ECOG PS 0 (0-2), hormonal receptor positive 152 pts (70%), 60 pts (27.6%) received neo/adjuvant chemotherapy (CT) + T and 75 pts (34.5%) adjuvant endocrine therapy. Most common metastatic sites: bone 121 pts (55.7%), liver 85 pts (39.2%), lung 61 pts (28.1%), soft tissues 133 pts (61.3%); 8 pts had CNS involvement. Seventy-eight pts (35.9%) had bone and/or soft tissues disease only; 108 pts (49.7%) had metastatic disease on presentation. Median number of metastatic sites was 3 (1-8). 144 pts (66.3%) and 72 pts (33.2%) received docetaxel (D) and paclitaxel (P) respectively. Median number of CT cycles was 6 for both drugs (D range 1-13; P range 1-18). Up to now 23 pts are still on CT and 86 on maintenance; ORR in evaluated pts (189) is 80.9% (47 and 106 pts obtained CR and PR respectively), 11 pts experienced PD during CT; 113 pts (58.2%) received endocrine therapy during maintenance. Median PFS is 14.7+ months (0.3+ - 53.2+). Most common adverse events are reported in table 1.

Table 1

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>All grades N (%)</th>
<th>Grade 3-4 N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukopenia</td>
<td>39 (18)</td>
<td>10 (4.6)</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>42 (19.3)</td>
<td>16 (7.3)</td>
</tr>
<tr>
<td>Anemia</td>
<td>50 (23)</td>
<td>2 (0.9)</td>
</tr>
<tr>
<td>Febrile Neutropenia</td>
<td>7 (3.2)</td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>103 (47.4)</td>
<td>10 (4.6)</td>
</tr>
<tr>
<td>Astenia</td>
<td>127 (58.5)</td>
<td>5 (2.3)</td>
</tr>
<tr>
<td>Peripheral Neuropathy</td>
<td>80 (36.8)</td>
<td>3 (1.3)</td>
</tr>
<tr>
<td>Stomatitis</td>
<td>67 (30.8)</td>
<td>2 (0.9)</td>
</tr>
</tbody>
</table>

Nail changes, nausea, alopecia, rash and arthro-myalgia were also reported. Three pts interrupted CT due to symptomatic drop of left ventricular ejection fraction (LVEF); 2 pts interrupted maintenance P due to rash and dyspnea respectively, 16 stopped P and T due to drop in LVEF, 1 due to occurrence of ALL and 1 refused to continue.
Conclusions: Our preliminary results highlight the activity and safety of the combination of CT plus P and T in unselected HER-2+ MBC patients. The study is ongoing and updated results will be presented.
Title: Retrospective review of palbociclib (Pal) efficacy and benefit from subsequent treatments following Pal progression in patients (pts) with hormone receptor positive (HR+) and HER2 negative (HER2-) metastatic breast cancer (MBC)

Jing Xi1, Aabha Oza1, Shana Thomas1, Michael Naughton1, Folusa Ademuyiwa1, Katherine N Weilbaecher1, Rama Suresh1, Ron Bose1, Mathew A Cherian1, Leonel Hernandez-Aya1, Ashley Frith1, Lindsay L Peterson1, Jairam Krishnamurthy1 and Cynthia X Ma1. 1Washington University School of Medicine, St. Louis, MO.

Body: Background
The cyclin-dependent kinase (CDK) 4/6 inhibitor Pal is approved for HR+ HER2- MBC. However, the optimal therapy following Pal progression is unknown. Therefore we conducted this retrospective study to review Pal efficacy and summarize the practice pattern and responses to subsequent treatments post Pal progression.

Methods
We performed a chart review of pts with HR+ HER2- MBC who began Pal treatment at Washington University Siteman Cancer Center between Feb 16, 2015 and July 13, 2016 and collected information on pts demographics, diagnosis, and treatment history. Duration of therapy was used to calculate the progression free survival (PFS) for each regimen. Treatment was considered first-line if administered without any prior systemic therapy or at least 1 year from completion of adjuvant hormonal therapy (HT). Treatments received after progression on 1st line therapy or upon relapse during or within 1 year from the completion of adjuvant HT were considered second-line regimens.

Statistical analyses were performed on SAS software, version 9.4. The Kaplan-Meier method was used to generate time-to-event curves, from which median PFS was calculated. A stratified log-rank test was used for all comparisons, and the P value derived from the comparison was reported.

Results
We completed a chart review for 81 pts (78 female and 3 male; 63 Caucasian, 14 African American, and 4 other races) with HR+ HER2- MBC (68 were ER+PR+, 13 were ER+PR-) who received Pal plus letrozole (n=65) or fulvestrant (n=15) or anastrozole (n=1), with a median age of 62.0 years (range 28.1 - 85.6) at the start of Pal.

The median follow up was 20.0 months (mos) (range 10.8 – 27.9). 25 pts were still on Pal treatment. The median PFS on Pal was 19.9 mos in the first-line setting (n=20), compared to 12.1 mos and 4.4 mos in the second-line (n=14) and subsequent lines (n=47), respectively (p=0.0287). Among the 54 pts who progressed on Pal, 38 moved on to the next treatment. 20 pts received chemotherapy and 16 pts received HT or a HT combination. 2 pts received fulvestrant plus Pal upon progression on letrozole plus Pal, and treatment was still ongoing at 4 mos and 7 mos of follow up, respectively. The most common treatments post Pal were single-agent capecitabine (Cape) (n=9) and the combination of exemestane (Exe) and everolimus (Eve) (n=8). The median PFS was 4.7 mos with Cape compared to 8.4 mos with Exe and Eve (p=0.60). The median PFS was 4.7 mos for the 20 pts who received chemo, whereas the median PFS was 4.9 mos with subsequent HT (n=16) (p=0.75).

Conclusion
Pal plus letrozole or fulvestrant is effective for the treatment of HR+ HER2- MBC, with activity observed beyond the 1st and 2nd line treatment settings. The PFS of Pal observed in this single center retrospective study is consistent with that of published data. Single-agent cape or the Exe and Eve combination were common treatment choices following progression on Pal. Although the study is limited by its small sample size, the median PFS of 8.4 mos with Exe and Eve indicates its potential efficacy in the setting of Pal progression. Additional pts and followup data will be presented.
Title: Treatment patterns and outcomes of pertuzumab in combination with trastuzumab and docetaxel as first-line treatment of metastatic HER-2 positive breast cancer: Comparison of Czech clinical registry and CLEOPATRA trial data

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Body: Key words: pertuzumab, HER-2 positive, breast cancer

Background: The present retrospective analysis was conducted to evaluate efficacy of pertuzumab, a monoclonal antibody, used in combination with trastuzumab and docetaxel as first-line therapy for metastatic HER-2 positive breast cancer in real-world clinical practice. The database of the Czech Clinical Registry of cancer patients treated with pertuzumab was used.

Materials and methods: Data of patients included in the national registry were analyzed and the outcomes were compared with the results of the Phase III CLEOPATRA trial (Baselga et al 2012). The registry is estimated to cover at least 95% of patients treated with the regimen outside of clinical trials.

Results: A total of 182 patients (mean age 56.5 years) were included in the present analysis. Patients had performance status of 0 (61.0%) or 1 (39%), 64.6% were postmenopausal, 62.6% had visceral disease and 36.8% received neo/adjuvant trastuzumab. The median progression-free survival of 21.2 months (95% CI 12.2–NR [not reached]) for patients included in the registry was longer, compared to 18.5 months reported in the CLEOPATRA trial, although the difference did not reach statistical significance (p=0.13). Best response was evaluable in 79.7% of patients. The overall response and disease control rates were 57.2% and 98.6%, respectively. Median overall survival has not been reached; the survival at 18 months was 86.6% (95% CI 75.7%-92.9%). Pertuzumab with trastuzumab and docetaxel was well tolerated with adverse events (AEs) attributed to pertuzumab reported in 8 patients. No AEs were life-threatening.

Conclusion: Pertuzumab in combination with trastuzumab and docetaxel is an effective and well-tolerated first-line therapy for patients with metastatic HER-2 positive breast cancer in real-world clinical practice setting. The PFS observed was consistent with data of CLEOPATRA trial.
Title: AZD5363 in combination with fulvestrant in AKT1-mutant ER-positive metastatic breast cancer

Lillian M Smyth1, Mafalda Oliveira2, Eva Ciruelos3, Kenji Tamura4, Anthony El-Khoueiry5, Alain Mita6, Benoit You7, Daniel J Renouf8, Marie-Paule Sabin9, Ana Lluch10, Ingrid A Mayer11, Hideaki Bando12, Hiroko Yamashita13, Helen Ambrose14, Elza de Bruin14, T Hedley Carr14, Claire Corcoran14, Andrew Foxley14, Justin PO Lindemann14, Rhiannon Maudsley14, Martin Pass14, Andrzej Rutkowski14, Gaia Schiavon14, Udai Banerji15, Maurizio Scaltriti1, Barry S Taylor1, Sarat Chandarlapaty1, José Baselga1 and David M Hyman1.

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Body: Background: E17K is the most common activating AKT1 mutation and was shown to be a therapeutic target in this multipart Phase 1 study of AZD5363 (NCT01226316), an oral and selective pan-AKT kinase inhibitor, in patients (pts) with AKT1-mutant (AKT1m) advanced solid tumors. In heavily pretreated AKT1m (E17K) ER+ metastatic breast cancer (MBC) pts, monotherapy achieved an objective response rate (ORR) of 20% and a median progression-free survival of 5.5 months (95% CI, 2.9–6.9). Suppression of PI3K-AKT signaling results in induction of ER-dependent transcription, potentially limiting the response to single-agent PI3K/AKT inhibitors. We explored the hypothesis that simultaneous inhibition of AKT and ER signaling would enhance antitumor efficacy in AKT1m ER+ MBC.

Methods: In an expansion of this study, we administered oral AZD5363 400 mg twice daily, 4 days on 3 days off, and fulvestrant 500 mg, to AKT1m (detected in tumor tissue by local screening and/or plasma BEAMing) ER+ HER2– MBC pts, enrolled into a fulvestrant-naïve (FN) or fulvestrant-resistant (FR) cohort (max 24 pts/cohort). Key objectives included safety and efficacy by RECIST v1.1. We report results of a planned interim analysis conducted when 12 pts/cohort reached maturity for assessment of 24-week clinical benefit rate (CBR), defined as the percentage of responders plus those with stable disease (SD) ≥24 weeks. Data cut-off occurred in June 2017.

Results: At the time of analysis, 24 AKT1m pts (23 E17K, 1 E40K) had received treatment. FN had more visceral disease (83.3% vs 66.7%) and ER+/PR– status (25% vs 8.3%) than FR. Median number of prior anticancer regimens was 4.5 (range 1–9) and 6 (2–11) in FN and FR, respectively, with more chemotherapy (CT) and less hormone therapy (HT) exposure in FN vs FR [3 (0–5) vs 2 (0–6) and 2 (0–4) vs 4 (2–6) prior CT and HT, respectively]. Prior palbociclib was received by 1 (8.3%) and 4 (33.3%) pts in FN and FR, respectively. Clinical efficacy is detailed below; CBR was 33% and 42% in FN and FR, respectively (Table 1). There was 1 unconfirmed partial response in patients treated with prior palbociclib and 3 SD. At data cut-off, 18 pts had discontinued treatment: progressive disease, n=12; adverse events (AEs), n=2; other reasons, n=4. AEs were observed in all 24 pts, most commonly diarrhea (71%), nausea (63%), vomiting and decreased appetite (29%). Grade ≥3 AEs occurred in 13 (54%) pts, most frequently maculopapular rash (n=3), nausea, hyperglycemia and back pain (all n=2). Dose reduction due to AEs occurred in 3 pts.

Table 1. Clinical efficacy

<table>
<thead>
<tr>
<th></th>
<th>FN</th>
<th>FR</th>
</tr>
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<tbody>
<tr>
<td>Eligible for interim data cut-off, n</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>ORR, n (%)</td>
<td>2 (17)</td>
<td>4 (33)</td>
</tr>
<tr>
<td>CBR, n (%)</td>
<td>4 (33)</td>
<td>5 (42)</td>
</tr>
<tr>
<td>Confirmed response (complete/partial response), n (%)</td>
<td>2 (17)</td>
<td>4 (33)</td>
</tr>
<tr>
<td>SD ≥24 weeks, n (%)</td>
<td>2 (17)</td>
<td>1 (8)</td>
</tr>
</tbody>
</table>
Conclusions: AZD5363 plus fulvestrant is clinically active in AKT1m ER+ MBC pts, including in pts with demonstrated prior resistance to fulvestrant. Comparatively lower efficacy was observed in the FN cohort; factors that may have potentially contributed (eg disease characteristics) will be explored. cfDNA and genomic data will also be presented.
Title: Evaluating the risk of upgrade to invasive breast cancer and/or DCIS on excision following a diagnosis of non-classic lobular carcinoma in situ

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Body: Introduction: Non-classic lobular carcinoma in situ (NC-LCIS) is a rare pathologic entity which encompasses a variety of histologic diagnoses. As such its natural history, including upgrade rates to invasive cancer (IC) or ductal carcinoma in situ (DCIS) on excision, is poorly characterized. We sought to evaluate the risk of upgrade to IC or DCIS when NC-LCIS is diagnosed on core biopsy.

Methods: After obtaining IRB approval, institutional pathology databases were searched for NC-LCIS core biopsy diagnoses (carcinoma in situ (CIS), carcinoma in situ with ductal and lobular features (CIS/DLF), pleomorphic LCIS (P-LCIS), variant LCIS (V-LCIS), LCIS with necrosis). Cases with a NC-LCIS core biopsy diagnosis and with available pathology results from subsequent surgery were included. Cases with known concurrent ipsilateral IC, DCIS and/or atypical ductal hyperplasia were excluded. Results: 107 cases with NC-LCIS in any pathology report were identified (1998-2016); 44 were excluded due to concurrent ipsilateral IC, the remaining 62 patients with 63 core biopsy diagnoses of NC-LCIS all underwent surgical excision and formed our study cohort. Median age was 56 years (range 43-83); 43 (68%) were postmenopausal. NC-LCIS was diagnosed on core biopsy for mammographic findings in 57 (90%) cases and for MRI findings in 6 (9%). All were BI-RADS 4 lesions; calcifications were the most common biopsy indication (50 (78%)). CIS/DLF was the most common term used for NC-LCIS (28 (44%)), followed by CIS (18 (29%)), V-LCIS (14 (22%)) and P-LCIS (3 (5%)). On core biopsy, 36/44 (82%) of NC-LCIS cases were E-cadherin negative, 38/41 (93%) were ER positive, and 6/34 (18%) were HER2 positive. IC and/or DCIS were diagnosed on subsequent surgery in 22 (33%) of patients, of which 14 (67%) were IC and 8 (18%) had DCIS only.

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Total</th>
<th>E-cadherin negative</th>
<th>Upgraded, N (%)</th>
<th>Invasive cancer, N (%)</th>
<th>DCIS only, N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIS</td>
<td>18</td>
<td>8/10 (80%)</td>
<td>3 (16%)</td>
<td>2 (67%)</td>
<td>1 (33%)</td>
</tr>
<tr>
<td>CIS/DLF</td>
<td>28</td>
<td>19/23 (83%)</td>
<td>12 (43%)</td>
<td>7 (58%)</td>
<td>5 (42%)</td>
</tr>
<tr>
<td>P-LCIS</td>
<td>3</td>
<td>1/1 (100%)</td>
<td>3 (100%)</td>
<td>2 (67%)</td>
<td>1 (33%)</td>
</tr>
<tr>
<td>V-LCIS</td>
<td>14</td>
<td>8/10 (80%)</td>
<td>4 (29%)</td>
<td>3 (75%)</td>
<td>1 (25%)</td>
</tr>
</tbody>
</table>

Median IC size was 0.2 cm (0.06-1.1 cm). IC histology was ductal in n=4 (29%), lobular in n=7 (50%), and ductal and lobular in n=3 (21%). Among the 14 invasive lesions, 5 (36%) were grade I, 5 (36%) were grade II and 2(13%) were grade III, (grade was not reported for 2 remaining ICs); 12/14 (86%) were ER positive and 1/14 (7%) was HER2 positive; none had LVI or positive nodes.

Among the 42 cases not upgraded, 13 (31%) had mastectomy, 9 (21%) had excision and radiation, 20 had excision only, all had negative margins. At median follow-up of 60 months (1-224 months), 1/20 patients treated with excision only was diagnosed with DCIS, 14 months after surgery for CIS/DLF on core biopsy.

Conclusions: In this large series of NC-LCIS diagnosed on core biopsy, the upgrade rate to carcinoma was 33% supporting the recommendation for routine excision of these lesions. The cancers found at excision were all stage I and the majority were grade I or II. At a median follow-up of 60 months only 1/20 patients with pure NC-LCIS treated with excision alone developed a future ipsilateral cancer. Further study of the natural history of these rare lesions is warranted.
Surgical strategy after neoadjuvant therapy in patients with operable breast cancer can be optimized by knowledge of the level of agreement of measured tumor size on MRI after neoadjuvant therapy and final pathologic assessment.

Body: Introduction
Neoadjuvant chemotherapy (NAC) is increasingly applied in the treatment of patients with operable breast cancer. Wide local excision after NAC aims to remove the complete residual tumor with no tumor on ink, without compromising cosmetics. Therefore, surgical strategies are determined based on the remaining tumor size after NAC visualized on magnetic resonance imaging (MRI). Recent studies on the correlation between preoperative MRI and pathological response demonstrate inconsistent results. A need remains to adequately guide surgical decisions after NAC. The aim of this study was to not only investigate the correlation between MRI and pathological evaluation but to gain knowledge of the exact level of agreement, specified for different tumor subtypes, which could further guide surgical decision making.

Methods
All patients operated for breast cancer after NAC between January 2013 and July 2016 in a large teaching hospital were retrospectively included. Longest residual tumor diameter was determined with MRI and correlated with postoperative pathological findings. Tumors were subdivided based on estrogen receptor (ER) status and human epidermal growth factor receptor 2 (HER2). Spearman correlation was used to correlate MRI and pathological tumor size findings. Bland-Altman method was used to evaluate the agreement between both measurements.

Results
193 patients with 195 breast cancers were included. The correlation between tumor size at MRI and pathology was 0.63 for the whole group, 0.39 for tumors with subtype ER+/HER2-, 0.55 for ER+/HER2+, 0.63 for ER-/HER2+ and 0.85 for ER-/HER2-. The correlation for lobular carcinomas was 0.44. The mean difference and limits of agreement (LoA), between tumor size measured at MRI and pathological size was 4.6 mm (LoA -27.0 to 36.3 mm, n=195). Mean difference and LoA for subtype ER+/HER2- was 7.6 mm (LoA -31.3 to 46.5 mm, n=100), for ER+/HER2+ 0.9 mm (LoA -8.5 mm to 10.2 mm, n=33), for ER-/HER2+ -1.2 mm (LoA -5.1 to 7.5 mm, n=21), for ER-/HER- -0.4 mm (LoA -8.6 to 7.7 mm, n=41) and 19.4 mm (LoA -16.8 to 55.6 mm, n=14) for lobular carcinoma.

Conclusion
The correlation and agreement between the post-NAC MRI and postoperative pathological assessment of residual tumor size for ER+/Her2- and lobular tumors is weak. The agreement shows a wide variation in over- and underestimation of tumor size in mm by MRI and thus surgical strategy can still be poorly guided. It does however provide us with exact information on the possible range of margins we should take into account at surgery. As demonstrated in other studies ER+/HER2+, ER-/HER2+ and ER-/HER2- tumors demonstrate a clear correlation. Also, the level of agreement in these tumor subtypes shows that we can use MRI evaluation as a reliable predictive tool of tumor residual size to base our surgical strategy upon with a small variation in over- and underestimation of tumor size. Because the lobular carcinoma and HER2+ subgroups were small, conclusions should of course be viewed cautiously.
Title: Arm node preserving surgery in primary breast cancer patients: 5 year experience

Jeong Yeong Park¹, Jung Eun Choi¹, Young Kyung Bae¹ and Soo Jung Lee¹. ¹Yeungnam University Medical Center, Daegu, Republic of Korea.

Body: Background:
Lymphedema is one of the major complications of axillary lymph node dissection (ALND) in patients with breast cancer. Axillary reverse mapping (ARM) is the technique to find lymphatic drainage from the arm during ALND. The purpose of this study is to evaluate the efficacy of arm node preserving surgery using ARM for reducing the incidence of lymphedema after axillary lymph node dissection in breast cancer patients and its oncologic safety.

Methods:
From January 2009 to October 2014, 167 patients with primary breast cancer were included. In all patients, 1 mCi of 99mTc-phytate was injected at the ipsilateral subareolar plexus and for axillary reverse mapping, 2.5mL of methylene blue was injected into the subcutaneous area of the medial intermuscular groove of the ipsilateral upper arm. The injection site was massaged for at least 5 minutes with the arm lifted above the heart level. At least 15 minutes later, ALND was performed and blue-stained arm nodes were identified. Arm nodes that were enlarged, hard or looked suspicious for metastasis were removed and all other arm nodes were preserved. Arm circumference at 10cm proximal to the medial epicondyle were measured pre- and post-operatively for 2 years. Circumference difference between both upper arms (CD) was evaluated and lymphedema was defined as CD of ≥2cm. Follow-up studies were performed every 6 months for 5 years and then annually using mammography, ultrasonography, and/or positron emission tomography.

Results:
Among 167 patients, 125 patients (74.9%) had their arm node preserved (ANP) and 42 (25.1%) patients had their arm node removed (ANR). Statistically significant difference in the mean number of harvested nodes was observed between ANP group (17.85±6.74) and ANR group (20.17±6.08) (p=0.05). The mean number of total identified blue stained arm nodes were 1.35±0.84. The mean follow-up period for measurement of arm circumference was 16.62±8.36 months. The last measured CD between both upper arms was 0.19±0.67cm in ANP group and 0.67±0.92cm in ANP group (p=0.003). 20 patients complained subjective symptoms of lymphedema, 7 patients in ANP group and 13 patients in ANR group (5.6% vs 31%, p<0.001). Among them, one patient in ANP group and 6 patients in ANR group were diagnosed with lymphedema (0.8% vs 14.3%, p=0.001). The other 13 patients’ CDs between both upper arms were below objective criteria of lymphedema. Follow-up studies were performed for 59.4±22.40 months. There were 16 cases of distant metastasis, 12 cases in ANP group, 4 cases in ANR group (9.6% vs 9.5%, p=1). Two patients in ANP group had distant metastasis and ipsilateral axillary recurrence simultaneously, but their TNM stages were already IIIC and IIb at the diagnosis. There’s no solitary axillary recurrence.

Conclusion:
Arm node preserving surgery using axillary reverse mapping in breast cancer patients can reduce the incidence of lymphedema after axillary lymph node dissection and it simultaneously has oncologic safety.
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Title: Efficacy of thoracic paravertebral block for reducing acute post-mastectomy pain

Fernando Herazo-Maya, Jorge Egurrola, Carlos E Restrepo, Lina Torres, Luis Palacios, Carlos A Ossa, Mauricio Borrero, Gonzalo A Angel, Juan J Marquez, Santiago Valencia, Alejandro Perez, Juan C Oyola, Laura Lobo, Rodolfo Gomez and Hector Garcia. 1Instituto de Cancerologia Clinica Las Americas, Medellin, Colombia; 2Clinica Las Americas, Medellin, Colombia and 3Universidad CES, Medellin, Colombia.

Body: Background: Mastectomy is associated with acute and chronic postoperative pain. This study investigated the efficacy of thoracic paravertebral block (TPB) compared to local anesthetic of surgical wound (LASW) in breast cancer patients undergoing mastectomy.

Methods: This phase 3, randomized controlled, single blind, parallel arms and superiority clinical trial aimed to evaluate the efficacy of TPB compared to LASW on acute pain at rest and motion at 24 hours post-mastectomy, measured by a visual analog scale (VAS). The study size of 60 patients is determined to have 90% power to detect a difference of 20% in acute pain in favor of TPB. In TPB group use of ultrasound was mandatory to guide puncture of intervertebral space T3 for infiltrating 0.5% bupivacaine at dose of 1.5 mg/kg. In LASW group was used bupivacaine 0.5% at dose of 1.5mg/kg on subcutaneous tissue of surgical area. VAS was used to measure surgical pain at rest and motion in 2,4,6,12 and 24 hours post-operatives for both groups. Trial Registry: ClinicalTrials.gov; Identifier:NCT02609321.

Findings: From 08-2015 to 09-2016, 60 breast cancer patients were enrolled; 3 dropped off consent. Mean age was 51 year; 78% had stage II and III; and 65% receive neoadjuvant chemotherapy. There were no significant differences in the VAS pain measurement for the groups of BWT compared to LASW in the 24 hour measurement, neither at rest (P=0.6525) nor in movement (P=0.7929). There were no significant differences in both groups for repeated pain measurements (5 measures mean), total dose of opioid administration, time to first dose of opioids or adverse events.

Conclusions: There were not statistical differences for post-mastectomy acute pain in first 24 hours between TPB and LASW patients. According our findings the use of TPB should not routinely recommended for breast cancer patients undergoing mastectomy.
Title: Evaluation of simplified lymphatic microsurgical preventing healing approach (SLYMPHA) for the prevention of breast cancer-related clinical lymphedema after axillary lymph node dissection

Tolga Ozmen¹, Mesa Lazaro¹, Yan Zhou¹, Alicia Vinyard¹ and Eli Avisar¹. ¹University of Miami, Miller School of Medicine, Miami, FL.

Body: Background: Lymphedema (LE) is a serious complication of axillary lymph node dissection (ALND) with an incidence rate of 16%. Lymphatic Microsurgical Preventing Healing Approach (LYMPHA) has been proposed as an effective adjunct to ALND for the prevention of LE. This procedure however requires microsurgical techniques. The aim of this study was to assess the efficiency of Simplified-LYMPHA (SLYMPHA) in preventing LE in a prospective cohort of patients.

Methods: All patients, undergoing ALND with or without SLYMPHA between January 2014 and December 2016 were included in the study. SLYMPHA is a slightly modified and simplified version of LYMPHA. It is performed by the operating surgeon performing the ALND. One or more lymphatic channels identified by reverse arm mapping are inserted using a sleeve technique into the cut end of a neighboring vein. During follow-up visits, tape-measuring limb circumference method was used to detect clinical LE. Demographic, clinical, surgical and pathologic factors were recorded. The incidence of clinical LE was compared between ALND with and without SLYMPHA. Univariate and multivariate analysis were used to assess the role of other factors in the appearance of clinical LE.

Results: 406 patients were included in the study. SLYMPHA procedure was attempted in 81 patients and was completed successfully in 90% of patients. Early complication rates were similar between patients who underwent SLYMPHA and who did not (4% vs. 4.13%; p = 0.948). Median follow-up time was 15±13.73 [1-32] months. Patients, who underwent SLYMPHA, had a significantly lower rate of clinical LE both in univariate and multivariate analysis (3% vs 19%; p = 0.001; OR 0.12 [0.03-0.5]). Excising > 22 lymph nodes and a co-diagnosis of diabetes were also correlated with higher clinical LE rates on univariate analysis, but only excising > 22 lymph nodes remained to be significant on multivariate analysis.

Conclusions: SLYMPHA is a safe and relatively simple method, which decreases incidence of clinical LE dramatically. It should be considered as an adjunct procedure to ALND for all patients during initial surgery.
Title: Less axillary lymph node dissections in patients with locally advanced breast cancer since implementation of the MARI-procedure

Catharina Boersma, Daniëlle Koopman-Kuin, José Van der Starre-Gaal, Jan H Korte, Ellen MA Roelofzen, Anne Brecht Francken and Eva M Noorda. 'Isala, Zwolle, Overijssel, Netherlands.

Body: Introduction
Over the last years patients with locally advanced breast cancer are increasingly treated with neoadjuvant chemotherapy (NAC). This can lead to downstaging of the breast tumor, leading to less extensive surgery and can also impact axillary tumor burden. In multiple reported series downstaging of axillary lymphogenic metastasis has been reported to complete pathological response (pCR) in 20-42% of patients. Radioactive iodine seeds have been used to mark the axillary metastatic node (MARI-procedure). This node is selectively removed after NAC. The need for additional surgery or radiotherapy of the axilla depends on the postoperative pathological outcome. This study shows the results of implementation of the MARI-procedure and its impact on postoperative axillary treatment.

Methods
A retrospective analysis done on outcome of the MARI-procedure in consecutive patients from 2015 - 2017 treated with NAC for stage III breast cancer in a large teaching hospital. Patients’ axillary status was determined pre-NAC by axillary ultrasonography, FNA and PET-scan. In these patients the metastatic lymph node was selectively marked with iodine seed. At surgery post-NAC the lymph node was removed. Adjuvant treatment was determined in the tumor board depending on the pathological result. Table 1 demonstrates the used algorithm for adjuvant axillary treatment. Descriptive statistics in terms of measured frequencies and central tendencies were used to describe the outcomes.

Table 1: adjuvant axillary treatment algorithm

<table>
<thead>
<tr>
<th></th>
<th>FNA proven axillary lymph node metastasis</th>
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<tbody>
<tr>
<td>N1</td>
<td></td>
</tr>
<tr>
<td>pCR</td>
<td>Residual tumor</td>
</tr>
<tr>
<td>No axillary treatment</td>
<td>Axillary radiotherapy</td>
</tr>
<tr>
<td>N2-3</td>
<td></td>
</tr>
<tr>
<td>pCR</td>
<td>Residual tumor</td>
</tr>
<tr>
<td></td>
<td>Axillary radiotherapy</td>
</tr>
<tr>
<td></td>
<td>ALND + axillary radiotherapy</td>
</tr>
</tbody>
</table>

Results
In total 50 consecutive patients were included and analyzed. Thirty one patients were staged cN1. Ten of these patients demonstrated a complete pathological response (ypN0) and did not receive any adjuvant axillary treatment. Sixteen patients had residual pathological tumour activity (ypN1) and received axillary radiotherapy. Five patients with residual disease underwent ALND (two patients ypN1, three patients ypN2). In the two patients staged ypN1, the algorithm was not followed due to progressive disease in the breast and the other because of occult axillary disease.Nineteen patients were staged cN2. Twelve of these patients had a complete pathological response (ypN0) and received axillary radiotherapy and seven patients had residual disease (ypN1 or ypN2) and treated with ALND. If ypN2 axillary radiotherapy was added. In total, 20% of patients did not receive any adjuvant axillary treatment, 56% received adjuvant axillary radiotherapy and 24% patients underwent ALND.

Conclusion
The number of patients with locally advanced breast cancer treated with ALND is obviously decreased since implementation of the MARI procedure after NAC. Hereby, one out of five patients could be spared any adjuvant axillary treatment. There is a need for follow up of long term results to assess recurrence risk and overall survival.
Title: Upgrade to high risk lesions, in situ and invasive cancer among women with benign papillary lesions diagnosed on image-guided core needle biopsy (IGCNB)

Gillian Kuehner¹, Jeanne A Darbinian², Sherry Butler³, Sharon Chang⁴, Louis Fehrenbacher¹, Rhona Chen³, Laurel A Habel² and Karen Axelsson⁵. ¹The Premanente Medical Group, Vallejo, CA; ²Kaiser Permanente Division of Research, Oakland, CA; ³The Permanent Medical Group, South San Francisco, CA; ⁴The Permanente Medical Group, Fremont, CA and ⁵The Permanente Medical Group, Oakland, CA.

Body: Background: Currently there is no consensus regarding the management of benign papillary breast lesions diagnosed on IGCNB. Recommendations vary as to whether all IGCNB papillary lesions require surgical excision or if IGCNB alone is adequate to confirm a benign diagnosis and patients can be followed with imaging.

Aims: To estimate percentage of patients with benign papilloma on IGBN who on surgical excision are upgraded to high risk lesion, in situ or invasive cancer and to identify patient, imaging, and/or pathologic features that are predictive of upgrade.

Methods: We conducted a study of 407 patients within Kaiser Permanente Northern California (KPNC) diagnosed with benign papillary breast lesions on IGCNB in 2012 and 2013. KPNC is a large integrated health care delivery system, racially and ethnically diverse, and representative of the underlying population. Patients were excluded from study if they were < 18 years, had atypia on IGCNB, had a prior history of breast cancer or high risk lesion, had a hereditary risk for developing breast cancer, or were noted to have papillomatosis or an incidental papilloma, or the target lesion was calcifications. Patients who did not have surgical excision of the IGCNB papilloma were followed for at least 2 years. Outcomes included in situ/invasive cancer and high risk lesions (atypical ductal or lobular hyperplasia, lobular carcinoma in-situ or papilloma with atypia). Outcomes were evaluated by review of medical records, including radiology, pathology, and surgical reports. The KPNC cancer registry and record review was used to exclude patients with a history of cancer.

Results: Among patients with benign papillary lesions, the average age was 56.4 years (range 20-93). Approximately 60% of lesions were 1 cm or less and 50% were centrally located (within 2 cm of nipple). There were 327 patients (80%) with surgical excision within 10 months of IGCNB, 61 patients (15%) with no surgical excision but follow-up imaging, and 19 patients (5%) with no surgery or follow-up imaging. Patients with and without surgical excision generally had similar age, breast density, and lesion location. However, surgical excision was more common among women with larger lesions. Among women with surgical excision, 9.5% (95% CI 6.3-12.7%) had a high risk lesion, 3.4% (95% CI 1.4-5.3-%) had an in situ lesion and 2.4% (95% CI 0.8-4.1%) had invasive cancer (all node negative). Less than 3% of women under 50 years, presenting with nipple discharge or with lesions less than 1 cm had invasive cancer on surgical excision. In contrast, over 10% of women with lesions greater than 1 cm, a palpable mass, or with lesions 5 or more cm from the nipple had invasive cancer on surgical excision. There were no cancers diagnosed among the 61 women followed by imaging; although 1 woman was upgraded to a high risk lesion.

Conclusions: In this large cohort of patients with benign papillary lesions on IGCNB, less than 3% had an invasive cancer on surgical excision. Upgrade was most common among patients with larger lesions, a palpable mass or lesions distant from the nipple and least common among women less than 50 years, with small lesions or presenting with nipple discharge.
Title: Fluorescence guided identification of tumor margins during breast conserving surgery

Chitresh Kumar, Anurag Srivastava, Piyush Ranjan, Kamal Kataria, Anita Dhar, Darakhshan Qaiser and Sandeep Mathur.
1All India Institute of Medical Sciences, New Delhi, Delhi, India.

Body: Background. Palpation guided breast conserving surgery for breast cancer is associated with tumor involved margins in up to 41% of cases. Positive margins warrant re-excision leading to higher disfigurement of breast, delay in adjuvant therapy and increased cost of treatment. Intraoperative ultrasound guidance and frozen section biopsy result in a significant reduction in margin involvement. However, these are not widely available in developing countries. Radio frequency wave (Margin Probe), and Raman spectroscopy had been recently investigated but were not founded to be cost effective and foolproof.

Novelty. We investigated the accuracy of a novel, cost effective and safe technique of margin assessment during breast conserving surgery.

Hypothesis. Intravenous Fluorescein achieves high concentration in tumor tissue due to increased capillary permeability which can be detected by blue light.

Objective. The aim of the present study was to determine whether fluorescence guided breast conserving surgery accurately identifies the tumor margins or not.

Methods.
Setting: All India Institute of Medical Sciences New Delhi, a tertiary care University teaching Hospital.
Study Design: Prospective Cohort study.
Study Period: Between March, 2016 to April, 2017.
A total of 52 breast cancer patients undergoing breast conserving surgery were included. Each patient received 2 ml of intravenous Fluorescein sodium (20%) just before skin incision. Breast conserving surgery was performed under ultrasound guidance. Specimen was bisected and examined under blue light. Six biopsies (1mm each) were obtained from non-fluorescent area 5mm away from fluorescent tumor margins at 2, 4, 6, 8, 10 and 12 o'clock position. All the non-fluorescent biopsies were evaluated for involvement by tumor on final histopathology.

Results. Mean age of the patient was 49.5 (S.D.=6.4 years). Mean size of the tumor on histology was 2.6 (S.D.=0.9 cm). Seventeen (32.7%) patients presented with T1 and 35 (67.3%) patients presented with T2 tumors. Four patients (7.6%) received chemotherapy before surgery. On bisecting the specimen, all tumors exhibited fluorescence under blue light. The non-fluorescent margins in two (3.8%) out of 52 patients were found to be involved by tumor on histology. Both the patients received chemotherapy before surgery. This technique of fluorescence guided margin assessment accurately predicted negative histological margin in 50 (96.2 %) out of 52 patients. None of the patients developed any adverse drug reactions to fluorescein.

Conclusion. Intravenous Fluorescein guidance is an effective and safe technique of margin assessment during breast conserving surgery.

Support. This study is funded by Council of Scientific and Industrial Research, India

References.
**Title:** Feasibility study to diagnose pathological complete response by neoadjuvant chemotherapy in breast cancer adding core needle biopsy (KBOG1301 supported by JONIE)

**Body:** Background: Neoadjuvant chemotherapy (NAC) for breast cancer has been improved and pathological complete response (pCR) achieved in about 50% of Her2 positive tumor and over 30% of triple negative tumor. It is possible that not to perform surgery if pCR is diagnosed accurately. This study was performed to examine diagnostic ability of core needle biopsy (CNB) to detect pCR by NAC. (Study registry number: UMIN000012035)

**Methods:** In this multicenter study, we registered histologically proved breast cancer patients who were diagnosed as clinical complete response (cCR) after NAC. The cCR was diagnosed by contrast-enhanced magnetic resonance imaging (MRI) after NAC by radiologists in each facility. Regimens of NAC were not specified by study protocol. A clip marker was not placed in the tumor before NAC. At the operating table, ultrasound-guided CNB is performed before starting the surgery. The CNB was performed with 14 gauge needle without vacuum assistance and requiring three biopsy specimens. The concordance of pathological results between a core needle biopsy and a surgical specimen is examined by pathologists at each facility. The pathological diagnosis was categorized as i) no carcinoma (pCR), ii) carcinoma in situ (CIS) and iii) invasive carcinoma.

**Results:** The study included 86 women from 10 facilities, accrued from December 2013 to March 2017. Median age was 53.5 (31-75) years and median tumor size before NAC was 2.3 (0.9-7.3) cm. Estrogen receptor was positive in 32 (37%). HER2 was positive in 40 (47%). The clinical stage before NAC was stage I 19 (22%), II 48 (56%), III 16 (19%) and IV 3 (3%). All cases were administered cytotoxic agents. Both anthracycline and taxane were given in 70 cases (81%), and trastuzumab was administered in 35 (41%) cases. As for breast surgery, partial resection was performed in 53 cases (62%), whereas 33 cases (38%) underwent mastectomy. Pathological examination on surgical specimen revealed pCR (i) in 41 cases (48%), CIS (ii) in 17 cases (20%) and invasive carcinoma (iii) in 28 cases (32%). Results of pathology of CNB and surgical specimens are shown in table 1.

<table>
<thead>
<tr>
<th>Surgical specimen</th>
<th>No carcinoma (pCR)</th>
<th>Residual carcinoma</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNB</td>
<td>41 (NPV=63%, spec.=100%)</td>
<td>24 (FNR=53%, 14 were CIS)</td>
<td>65 (76%)</td>
</tr>
<tr>
<td>Residual carcinoma</td>
<td>0</td>
<td>21 (PPV=100%, sens.=47%)</td>
<td>21 (24%)</td>
</tr>
<tr>
<td>Total</td>
<td>41 (48%)</td>
<td>45 (52%)</td>
<td>86 (100%)</td>
</tr>
</tbody>
</table>


The kappa value was 0.455 which was not enough for accurate diagnosis. In 24 discordant cases, residual tumor was found in surgical specimen but not in CNB, residual disease was CIS in 14 cases.

**Conclusions:** Up to 10% false negative rate of pCR may allow us to proceed to an observational study without performing surgery. This study revealed that the ultrasound-guided CNB for cCR cases by MRI was not accurate enough to omit surgery. Thus, imaging diagnosis and biopsy methods need to be improved. However, it is still possible to consider because the post-operative radiotherapy may compensate for surgery in cases with CIS after NAC.
Title: Intraoperative ultrasound guided surgery after neoadjuvant treatment in breast cancer improves patient’s quality of life

Isabel T Rubio¹, Roberto Rodriguez¹, Antonio Esgueva-Colmenarejo¹, Mireia Suarez¹, Martin Espinosa-Bravo¹, Christian Siso¹, Ocativi Cordoba¹, Stanley Alvarado¹, Jose Volders² and Petroujska Van Den Tol². ¹Hospital Univeristario VAll d’Hebron, Barcelona, Spain and ²VU University Medical Center, Amsterdam, Netherlands.

Body: Background: Intraoperative ultrasound guided surgery (IOUS) has shown not only to improve surgical outcomes by achieving higher rates of negative margins and reducing the need for re-excisions, but also showing better cosmetic results and patient's satisfaction in the adjuvant setting. After neoadjuvant treatments (NAT) we have shown that IOUS lowers the volume of resection in patients with pathologic complete response or minimal microscopic disease after NAT without compromising margins and local recurrences when compared to wire localization techniques (WL). The aim of this study was to determine if IOUS after NAT contributes to improve cosmetic outcomes and quality of life (QOL) when compared with WL.

Material and Methods. The pilot study enrolled patients treated with NAT who underwent breast-conserving surgery (BCS) between July 2008 and December 2012. On the follow up visit, cosmetic outcomes were assessed by the patient and surgeon on a 4 point Likert scale, by the Breast Cancer Conservative Treatment cosmetic results (BCCT.core) software, and by an independent panel. QOL was assessed using cancer-specific (EORTC QLQ-C30 and EORTC QLQ-B23) questionnaires.

Results. The study investigated 113 patients: 81 (71.6 %) in the IOUS group and 32 (28.3 %) in the WL group. The patient and tumor characteristics were comparable between the two groups. Mean follow up was 64 months (range, 12-90) in the WL group while 41 months (range, 18-107) in the IOUS group.

Cosmetic outcomes reported by the patient showed a tendency (p=0.07) for better results in the IOUS group. Patient reported cosmetic outcomes and the independent panel cosmetic evaluation significantly correlated with lumpectomy volume excised (p=0.04, p = 0.02 respectively). The BCCT.core did not correlate with volume of tissue excised (p < 0.14). IOUS achieved better cosmetic outcomes compared to WL, with excellent outcomes of 30% and 19% respectively, although poor cosmetic outcomes were 3% and 0% respectively. Correlation between patients and surgeons evaluation regarding cosmetic outcomes and satisfaction was low (0.38 and 0.40 respectively). A comparison of the QOL scores of 113 patients showed a significantly better global health status (p = 0.03), better emotional and role function (p = 0.004), for patients with IOUS compared to WL patients.

Conclusions. This is the first study to show that breast volume excised significantly correlates with cosmetic outcomes in the neoadjuvant setting. These results suggest that IOUS after NAT contributes to a better quality of life and may influence cosmetic outcomes in breast cancer patients. Patient reported outcomes should be included in the quality assessment in breast surgery as the correlation with surgeons’ evaluation is low.
Title: Enhanced intraoperative breast specimen assessment may reduce margin positivity and reoperation rates in breast cancer

Hanh-Tam Tran\textsuperscript{1}, Ilyssa Moore\textsuperscript{1}, Niya Patel\textsuperscript{1}, Shawna Willey\textsuperscript{2} and Maen Farha\textsuperscript{1}. \textsuperscript{1}Medstar Union Memorial Hospital, Baltimore, MD and \textsuperscript{2}Medstar Georgetown University, Washington DC.

Body: Introduction: Partial mastectomy is the most commonly performed operation for breast cancer. Margin positivity throughout the nation ranges from 30-60\%, of which 10-36\% require reoperation. Data from the SHAVE trial showed that excising an additional 1 cm margin circumferentially can reduce margin positivity by 50\%. We are concerned about the large volume of tissue that may be unnecessarily removed secondary to routine circumferential margin excision. We have practiced selective additional margin excision based on enhanced intraoperative margin assessment including gross specimen evaluation by pathology. We suggest that enhanced intraoperative specimen assessment may reduce margin positivity and reoperation rates.

Methods: This is a retrospective review of a prospectively maintained, single surgeon database of patients undergoing partial mastectomy between February 2014 – December 2016 at Medstar Union Memorial Hospital. One hundred consecutive patients diagnosed with DCIS or invasive carcinoma undergoing partial mastectomy with the intention of margin negativity were included. Information regarding preoperative planning and intraoperative specimen assessment were collected from clinical notes and operative records. These data points included preoperative imaging studies, use of preoperative needle localization, intraoperative ultrasound to guide surgery, use of intraoperative Faxitron, and gross pathologic consultation. The data is analyzed to calculate margin positivity and reoperation rates in comparison to reported experiences.

Results: The average age was 62.3 years, ranging from 33-96 years. Of the 100 patients, 73 had invasive cancer, 19 had DCIS. The average lesion size was 14.17mm. There were 8/100 cases with a positive margin, of which 5 cases had re-excisions. An additional 3 cases had re-excision for positive cells <1mm from margin. Faxitron was used in 89\% of cases and gross consultation in 100\% of cases. The average number of additional margins taken was 1.2. 22/100 cases did not have additional margins taken during the index operation.

Discussion: This is a hypothesis generating study that was prompted by our concern about the amount of tissue and the expense created by routine circumferential margin removal. The issue of variability among different institutions and the very high reoperation rates reported by many beg for an explanation. Many techniques have been proposed to reduce this unexplained variability but none has consistently shown the necessary improvement and many new technologies are costly. Our experience suggests that low margin positivity and reoperation rates may be achieved using inexpensive enhanced intraoperative specimen assessment. We can test this hypothesis by comparing information among the different hospitals in our system. A head to head prospective trial comparing routine shaves versus enhanced specimen assessment and selective additional margins would answer this question.

Conclusion: Enhanced intraoperative margin assessment and selective margin excision may provide a good alternative to routine shave margin while removing less breast tissue. Uniform specimen assessment algorithms may also help achieve reduced margin positivity and reoperation rates.
Title: Oncological safety of nipple-areola sparing mastectomy in comparison with skin sparing and total mastectomy: Results from a NCI-designated comprehensive cancer center

Zeynep Bostanci¹, Xinguang Wang², Rebecca Ottesen¹, Janet Nikowitz¹, Veronica C Jones¹, Linda Springer¹, Lily Lai¹, Lesley Taylor¹, Courtney A Vito¹, Isaac B Paz¹, Joyce Niland¹, Laura Kruper¹ and John H Yim¹. ¹City of Hope National Medical Center, Duarte, CA and ²Breast Cancer Unit, Peking University Cancer Hospital & Institute, Beijing, China.

Body: Nipple-areola sparing mastectomy (NSM) may be offered to some women with breast cancer as an alternative to skin sparing (SSM) or total mastectomy (TM) with excellent cosmetic results and acceptable recurrence risk. The aim of this study is to determine the local/regional recurrence rate of NSM in comparison to SSM and TM at our institution and to determine the factors that may be associated with risk of recurrence. Women who underwent NSM (n=148), SSM (n=660) or TM (n=443) at City of Hope National Medical Center between May 2007 and December 2014 for Stage 0-III breast cancer were identified retrospectively. Exclusions were: women with inflammatory breast cancer and those who had mastectomy for recurrent breast cancer. Overall survival (OS) and disease free survival (DFS) were analyzed using Cox regression controlling for age, race/ethnicity, stage, histology, grade, hormone receptor and Her2 receptor status. There were total of 165 NSMs, 704 SSMs and 466 TMs performed for cancer, accounting for the patients with bilateral cancers. The median follow up time was 38, 58 and 55 months for NSM, SSM and TM, respectively. Median (range) age at diagnosis was 49 (23-74) for NSM, 51 (23-90) for SSM and 59 (26-92) for TM. In the NSM group, 76% of patients had invasive ductal cancer (IDC) and 15% had ductal carcinoma in-situ (DCIS); this was comparable to 73% and 13% in the SSM group and 78% and 9% in the TM group, respectively. The majority of patients who underwent NSM had Stage II disease (45%), which was similar to SSM (43%) and TM (44%). Only 3% of NSM patients had Stage III disease compared to 17% of SSM patients and 29% of TM patients. Most of the patients in all 3 surgical groups received adjuvant chemotherapy (NSM 59%; SSM 52%; TM 51%). Of patients who underwent NSM, 20% received neoadjuvant chemotherapy, compared with 29% of SSM patients and 35% of TM patients. The local/regional recurrence rate per breast was 12/165 (7.3%) for NSM, 23/704 (3.3%) for SSM and 11/466 (2.4%) for TM (n=11). Median time to recurrence was 20, 26 and 16 months for NSM, SSM and TM, respectively. Of the NSMs performed only 1 recurrence occurred at the nipple-areolar complex (0.6%), 9 recurrences were at the chest wall (5.5%) and 2 were at the axilla (1.2%). Eight recurrences after NSM had DCIS in addition to IDC at the time of initial diagnosis while 2 had pure DCIS, 1 had pure IDC and 1 had invasive lobular cancer. There were 8 recurrences with estrogen receptor (ER) and progesterone receptor (PR) positivity at the time of initial diagnosis, that converted to ER+, PR-. One third of recurrences after NSM had multifocal disease. There was no significant difference found in adjusted overall survival (p=0.49) and adjusted disease free survival (p=0.10) among NSM, SSM and TM patients. Even though there is higher rate of local/regional recurrence with NSM, there is no difference in overall and disease-free survival at our institution. Presence of DCIS may be an important factor for recurrence. From these data we conclude that NSM is an oncologically acceptable alternative to SSM and TM, with excellent cosmetic results.
2017 San Antonio Breast Cancer Symposium

Publication Number: P5-22-13

Title: Representation of surgical breast research at an interdisciplinary oncology conference

Rebecca A Shuford1, Caleb R Dulaney1 and Audrey Wallace1. 1University of Alabama at Birmingham, Birmingham, AL.

Body: Background: The treatment of breast cancer requires an interdisciplinary approach, involving the fields of surgery, radiation oncology, medical oncology, pathology and radiology. The roles of various specialties may differ in the local versus metastatic setting. Despite the importance of collaborative care, significant disparities remain in federal funding of research among different medical specialties. The purpose of this analysis is to evaluate the representation of the surgical specialty at an interdisciplinary oncology research conference.

Methods: All electronically available abstracts in the breast cancer local, regional, adjuvant or breast cancer metastatic categories at American Society of Clinical Oncology (ASCO) 2017 annual meeting were reviewed. All abstracts containing the word “breast” in the title of other relevant categories were also included. These categories included developmental therapeutics, patient and survivor care, brain metastases, cancer prevention/hereditary genetics/epidemiology, tumor biology, health services/clinical informatics/quality of care. Only abstracts in these categories that were presented via poster or oral presentation at the annual meeting were included. The medical discipline, gender, and country of institution were recorded for each first and last author. Country of institution was recorded based on abstract reported affiliation. Gender and medical discipline were recorded based on information from academic and industry profiles available online.

Results: A total of 301 breast cancer posters (n = 276) and oral abstracts (n = 25) were presented at ASCO 2017. The majority of first (56.8%) and last (56.1%) authorships were from institutions in the United States. Geographic distribution of surgical first authors was as follows: 50.0% United States, 13.9% Europe and 36.1% Asia. While medical oncology represented the majority of first (59.5%) and final authorships (61.8%), surgery was underrepresented at 12.0% of first and 9.0% of final authors. When only non-metastatic breast cancer related abstracts were included (n = 142) surgical representation improved for both first (18.3%) and final (14.1%) authorship. While low in comparison to medical oncology, representation for the field of surgery was better than that of radiation oncology (2.3% first and 2.0% last authors), pathology (2.7% first and 3.3% last authors), and radiology (1.7% both first and last authors). In the entire cohort, female researchers were well represented as first authors (50.8%), but less often as final authors (41.9%). Representation of female surgeons was variable based on authorship: 61.1% of surgical first authors were female, but only 37% of surgical final authors were women.

Conclusions: The treatment of breast cancer requires a multidisciplinary approach. However, despite the collaborative nature of care, researchers in the field of surgery were less likely to present scholarly activity at ASCO 2017. This is likely multifactorial, and warrants further research to identify barriers and facilitators of surgical participation in the conduct and reporting of breast cancer surgical research.
**Title:** Assessment of risk factors in women undergoing contralateral prophylactic mastectomy after breast cancer diagnosis: Experience at an academic medical center

Anastasia Drobysheva¹, Yasmeen Butt¹ and Sunati Sahoo¹. ¹University of Texas Southwestern Medical Center, Dallas, TX.

**Body:** Recently, the rates of contralateral prophylactic mastectomy (CPM) have increased significantly with no clear evidence of improvement in overall survival or breast cancer (BC) specific survival. In 2016, American Society of Breast Surgeons (ASBS) provided a consensus statement regarding when CPM should be-, can be- or may be offered and when it should be discouraged. In light of new recommendations, our study aimed to review factors that influenced the choice of CPM at our institution.

**Methods:** This retrospective study was approved by the IRB. Patients who underwent CPM between January 2011 and May 2014 were included. Medical records were reviewed for documentation of risk factors that led to CPM. Patients were stratified into four categories based on the consensus statement. CPM “should be considered” included carriers of BRCA1/2 mutation, patients with strong FH (2 or more first degree relatives with BC) but not tested for BRCA and patients with history of mantle radiation; CPM “can be considered” included patients with intermediate FH (one first degree or multiple second/third degree relatives with BC), carriers of non-BRCA gene mutation, patients with prior history of BC and patients with strong FH but tested negative for BRCA; CPM “may be considered” included patients with psychological factors, those who denied adjuvant therapy and patients with multicentric disease in the index side. CPM “should be discouraged” included patients with advanced disease at the time of diagnosis, patients with weak FH (one second or third degree relative with BC) and patients with no significant FH or unknown FH.

**Results:** Between January 2011 and May 2014, CPM was performed in 261 women ranging from 24 to 83 years (mean, 50 years). The number and percentage of women in each risk group is summarized in Table. Discussion: Based on the results, only 13% of the 261 women satisfied the criteria for when CPM “should be considered”, 38% for when CPM “can be or may be considered”, and in the remaining 49% CPM should have been discouraged. In light of the growing controversy regarding the overall benefit of CPM, it is important that the women with average-risk are well informed about the surgical risks vs. the benefits of CPM.

**Summary of the risks factors associated with contralateral prophylactic mastectomy (n=261)**

<table>
<thead>
<tr>
<th>CPM should be considered for those at significant risk of CBC (total 13%)</th>
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<tbody>
<tr>
<td>Carriers for BRCA 1/2 mutation</td>
<td>29</td>
</tr>
<tr>
<td>Strong Family history (patient not tested for BRCA)</td>
<td>2</td>
</tr>
<tr>
<td>History of mantle radiation</td>
<td>3</td>
</tr>
</tbody>
</table>

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<tr>
<th>CPM can be considered for those at lower risk of CBC (total 30.6%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Intermediate family history</td>
<td>60</td>
</tr>
<tr>
<td>Carriers for non-BRCA gene mutation</td>
<td>3</td>
</tr>
<tr>
<td>History of prior breast carcinoma</td>
<td>13</td>
</tr>
<tr>
<td>Strong Family history, BRCA negative</td>
<td>4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CPM may be considered for other reasons (total 7.6%)</th>
<th></th>
</tr>
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<tbody>
<tr>
<td>Psychological factors</td>
<td>6</td>
</tr>
<tr>
<td>Patient denied adjuvant chemo or radiation therapy</td>
<td>3</td>
</tr>
<tr>
<td>Multicentric disease in the index breast</td>
<td>11</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CPM should be discouraged (48.8%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Advanced disease at diagnosis</td>
<td>4</td>
</tr>
<tr>
<td>Weak family history</td>
<td>78</td>
</tr>
<tr>
<td>No family history</td>
<td>41</td>
</tr>
<tr>
<td>Unknown family history, no other significant personal history</td>
<td>5</td>
</tr>
</tbody>
</table>

CPM: contralateral prophylactic mastectomy; CBC: contralateral breast cancer
Title: Hormone receptor status is a predictive factor for axillary lymph node recurrence after sentinel lymph node biopsy

Chikako Sekine¹, Satoko Nakano¹, Akemi Mibu¹, Masahiko Otsuka² and Toshinori Oinuma³. ¹Kawaguchi Municipal Medical Center, Kawaguchi, Saitama, Japan; ²Kawaguchi Municipal Medical Center, Kawaguchi, Saitama, Japan and ³Kawaguchi Municipal Medical Center, Kawaguchi, Saitama, Japan.

Body: Background
Axillary staging is important for predicting prognosis, and for local control in early breast cancer. Sentinel lymph node biopsy (SLNB) is a widely accepted method to avoid unnecessary axillary lymph node dissection (ALND). Since the ACOSOG Z0011 trial was published, we have refrained from ALND for selected patients with positive SLNB results. However, some cases have shown regional lymph node recurrences after SLNB without axillary dissection. The purpose of this study is to identify risk factors for recurrences, to ensure a safe axillary surgery.

Methods
A retrospective review of 1011 patients who underwent SLNB without ALND between June 2004 and March 2017 was performed. Since October 2012, we have not performed ALND in patients (a) with 1 or 2 positive sentinel lymph nodes (SLNs), (b) with positive SLNs that are unmatted or not gross extra nodal extension, (C) in whom clinical tumor size is <5 cm, and (d) who receive adjuvant endocrine therapy or chemotherapy and radiotherapy. Cases of mastectomy, lumpectomy with a positive margin and additional resection or boost radiotherapy, and bilateral cancer were included. SLNs were identified using technetium sulfur colloid and indigo carmine blue dye, and were bisected in parallel to the long axis of the nodes. The sections were stained with hematoxylin and eosin. Adjuvant systemic and/or radiation treatment was delivered as per the National Comprehensive Cancer Network and the Japanese Breast Cancer Society clinical practice guidelines and was based on the patients' pathological and clinical traits.

Results
Of the 1011 patients, 969 had negative and 42 had positive SLNs. The median age of patients was 59 years (range 21-88). The median invasive breast tumor size was 15 mm (range 0.05-85), with 1.9% tumors being pathological T3 lesions; 127 patients (12.3%) developed lymphatic vessel invasion. SLNs identification rate was 99.4%. The median number of SLNs removed per patient was 2 (range 1-7). After follow-up of a median 78.5 months, 10 patients (1.0%) had an axillary recurrence and all of them had negative SLN metastasis. The median time to axillary recurrence was 26 months (range 9-94). The hormone receptor (HR) status was significantly related to axillary recurrence (p=0.008). While triple negativity had a tendency to relate (p=0.06), human epidermal growth factor receptor 2 (HER2) status did not correlate with axillary recurrence (p=0.13).

<table>
<thead>
<tr>
<th>Tumor subtype</th>
<th>SLNB without ALND (n=1011)</th>
<th>Axillary recurrence</th>
<th>P value</th>
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<tbody>
<tr>
<td>HR positive</td>
<td>726</td>
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</tr>
<tr>
<td>HR negative</td>
<td>161</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>HER2 positive</td>
<td>121</td>
<td>3</td>
<td>0.13</td>
</tr>
<tr>
<td>HER2 negative</td>
<td>766</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Triple Negative</td>
<td>100</td>
<td>3</td>
<td>0.06</td>
</tr>
<tr>
<td>Not Triple Negative</td>
<td>787</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>DCIS</td>
<td>124</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

DCIS: Ductal carcinoma in situ

Conclusions
As reported previously, the axillary recurrence rate after SLNB was low. Our results show that HR negativity was a significant
factor for axillary recurrence. Although the ACOSOG Z0011 trial criteria focused on ALNB positive cases, they do not mention the tumor subtypes. Our findings show that HR negative patients without ALND have to follow up carefully.
Choosing wisely recommendations against prophylactic bilateral mastectomy: Analysis of the National cancer database

Audrey S Wallace¹, Abby Threet¹, Joshua Richman¹, Rachel Lancaster¹ and Catherine C Parker¹. ¹University of Alabama Birmingham.

**Background:** Surgical treatment for breast cancer is often a preference sensitive decision. The American Society of Breast Surgeons 2016 Choosing Wisely recommendation discourages routine contralateral prophylactic mastectomy (CPM). The purpose of this abstract is to analyze trends and factors that may be contributing to increasing utilization of CPM in the setting of unilateral breast cancer in the National Cancer Database (NCDB) from 2004-2013.

**Methods:** The NCDB is a national database of the American Cancer Society and the Commission on Cancer comprising approximately 70% of the cancer population in the US. Data from women with AJCC Stage 0-2 breast cancer treated with unilateral mastectomy (UM) vs CPM were abstracted. CPM was the primary outcome variable. Other variables included age, race, geographic region, payer status, income quartile, estrogen/progesterone receptor(ER/PR) status. HER2 status was not evaluated given limited availability of data. Categorical data was compared using Chi square tests. Odds ratios (OR) and Hazard ratios (HR) with confidence intervals (CI) were reported for univariate logistic regression models to evaluate effect of various factors on type of surgical treatment, and cox regression for survival with CPM, respectively. Significance was defined as p<0.01.

**Results:**

In the entire cohort, CPM was performed in 6.9% of ER/PR-, and 5.5% of ER/PR+ women (p<0.01). ER/PR + women were less likely to undergo CPM (HR 0.7, 95% CI 0.69-0.73, p< 0.01). Despite undergoing fewer CPM, women with ER/PR+ disease had improved overall survival in comparison to women who were ER/PR- There was an increase in utilization of CPM from 3.3% in 2004 to 7.5% in 2013 (p<0.01) Progressive year of diagnosis (HR 1.12, 95% CI 1.12-1.13, p<0.01) was associated with increasing treatment with CPM, but among newly diagnosed women with CPM there was no change in survival over time (HR 1.0).

**Conclusion:**

Women with newly diagnosed unilateral breast cancer are increasingly undergoing contralateral prophylactic mastectomy. There is great heterogeneity in socioeconomic factors associated with CPM. Women with ER/PR+ disease are undergoing CPM more often despite good outcomes in comparison to women with hormone negative disease. Given the new AJCC staging emphasis on tumor biology and disease outcomes, further research should consider socioeconomic variables as well as biology to create individualized risk assessment and decision aids to guide surgical decision making.
Body: Background: Breast lesions of uncertain malignant potential (B3) are a heterogeneous group of abnormalities with a variable but low risk of associated malignancy (7-46% in literature). The evidence base for appropriate management of B3 lesions in the breast is limited. The aim of our study was to determine breast cancer rate associated with B3 lesions diagnosed by either core needle biopsy (CNB) mostly using a 14G spring-loaded CNB or by vacuum-assisted biopsy (VAB) using a 7G–11G device. Secondary objectives were to determine predictive factors of cancer and evaluation of predictive models previously reported.

Material: Between January 2008 and December 2015, radio-histo-clinic criteria for patients with B3 lesions included in this prospective multicentric study (9 centers) were recorded in order to determine breast cancer rate, histologic type (infiltrative or in situ) according to spectrum of histological diagnoses including atypical ductal hyperplasia (ADH), flat epithelial atypia (FEA), classical lobular neoplasia (LN), papillary lesions (PL), benign phyllodes tumors (PT), and radial scars (RS). Patients with two or more histological B3 lesions were classified according to the most « pejorative » lesion.

Results: Among 518 patients, 86.3% were operated and 13.7% non-operated. Breast cancer rate was 15.3% with respectively 79.4% and 10.3% of in situ and infiltrative cancer. Criteria significantly correlated with surgery were: residual micro-calcifications after biopsy (p=0.005), histological type (Odd Ratio: 5 for ADH, 10 for RS, 6.5 for PL). Predictive factors of residual micro-calcifications after biopsy were: radiologic size > 10mm (p=0.002), size device ≥ 11 G (p=0.009). According to B3 histologic type breast cancer rates were: 12.9% for FEA, 20% for ADH, 11.6% for LN, 3.7% for RS and 8.8% for PL. Predictive models previously reported were not validated in our population.

Conclusion: About 80% of breast cancers associated with lesions of uncertain malignant potential were in situ. We propose an algorithm to decision help in order to propose complementary surgery or surveillance after B3 biopsy diagnosis.
Title: Nipple sparing mastectomy in unselect patients: Experience of an academic center in the south of Brazil

Antonio Frasson, Felipe Zerwes, Alessandra Souza, Nathalia Rossato, Isabela Miranda, Vanessa Trindade, Betina Vollbrecht, Marcelle Santos and Janaina Viegas. 'Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brazil.

Body: INTRODUCTION: Nipple sparing mastectomy (NSM) consists in the complete excision of the breast gland, maintaining the subcutaneous tissue, skin and nipple-areola complex (NAC). Recently, the NSM was developed as a therapeutic option not just for the patients that need risk-reducing surgery, but also for patients that have tumors not involving the NAC.

OBJECTIVES: Evaluate the overall survival, disease-free survival and local recurrence of the patients that underwent NSM. The only exclusion criteria for NSM was lesion at the NAC.

MATERIAL AND METHODS: Retrospective study that evaluate the number of cases of NSM performed between 2003 and 2015 in a private sector in the south of Brazil.

RESULTS: A total of 224 patients underwent NSM during the period of 2003-2015. The mean age was 47 years. From the total, 43 patients underwent risk-reducing NSM and 181 therapeutic NSM (64 unilateral and 117 bilateral). In those cases of bilateral procedures all of them underwent risk-reducing surgery on the contralateral breast (there were no bilateral cancers). Of this 181 cases, 108 (59%) have familiar history of breast cancer, considering first, second and third-degree relatives. Twenty six patients (14%) have history of previous breast cancer diagnosed. In this group of 181 patients with therapeutic NSM, 29 (16%) developed any unfavorable outcomes, 13 (7%) local recurrence, 4 (2%) ipsilateral node recurrence, 3 (1.6%) contralateral recurrence and 9 (5%) distant metastasis. None of the local recurrences occurred at the NAC, neither the cited unfavorable outcomes occurred in the risk-reducing setting. In a median follow-up of 47.7 months, the overall survival was 95% (8 deaths related to breast cancer) and the disease-free survival was 83.7%.

CONCLUSIONS: The overall survival rates of 95% at 47 months of follow-up is similar to the literature rates, that range from 93 to 97% in 5 years, depending on study's inclusion criterias. In our study, 14% of patients had previous breast cancer and 59% of patients treated for breast cancer by NSM had positive familiar history of breast cancer. This results in an unselected group of patients confirms the safety of this therapeutic method as an option for breast cancer patients.
2017 San Antonio Breast Cancer Symposium

Publication Number: P5-22-20

Title: Oncologic safety of endoscopic assisted breast surgery compared with conventional breast surgery: An analysis of 1295 primary operable breast cancer patients from single institute

Hung-Wen Lai¹, Shou-Tung Chen¹, Dar-Ren Chen¹ and Shou-Jen Kuo¹. ¹Changhua Christian Hospital, Changhua, Taiwan.

Body: **Background:** Endoscopy-assisted breast surgery (EABS) performed through minimal axillary and/or periareolar incisions is a possible alternative to open surgery for certain patients with breast cancer. In this study, we report the oncologic safety results of EABS compared with conventional breast surgery (CBS).

**Methods:** The medical records of patients who underwent EABS for breast cancer during the period June 2010 to April 2017 were collected from the EABS database at Changhua Christian Hospital, a tertiary medical center in central Taiwan. Data on clinicopathologic characteristics, type of surgery, method of breast reconstruction, margin involvement, locoregional recurrence, distant metastasis and overall survival were collected and compared to another cohort of patients, who received CBS at the same hospital, to determine the effectiveness and oncologic safety of EABS.

**Results:** A total of 1295 patients were enrolled in current study, including 214 patients receiving EABS and 1081 patients underwent CBS. The mean age of them were 50.4 ± 9.4 years-old in EABS group, and 52.5 ± 11.3 in CBS group (P=0.01). Patients who received EABS were associated with more early stage breast cancer than patients with CBS group (P<0.01). The margin involved rate was 3.3% in EABS group, and 6.1% in CBS group (P=0.11). During a median follow-up of 57.7 ± 24.7 months, the locoregional recurrence was 3.7% in EABS group, and 7.3% in CBS group (P=0.06). The distant metastasis rate was 2.8% in EABS group and 10.3% in CBS group (P=0.001). The overall survival rate was 99.5% in EABS group, and 93.8% in CBS group.

**Comparison of endoscopic assisted breast surgery (EABS) and conventional breast surgery (CBS)**

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<th>EABS</th>
<th>CBS</th>
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</tr>
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<tbody>
<tr>
<td>N=214 (%)</td>
<td>N=1081 (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pathology tumor size (cm)</td>
<td>2.2 ± 1.6</td>
<td>2.3 ± 1.7</td>
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<tr>
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<tr>
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<tr>
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<td>73 (34.1)</td>
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<td></td>
<td></td>
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<td>534 (49.4)</td>
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<td></td>
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<tr>
<td>Not done</td>
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<td>44 (4.1)</td>
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<td>Breast reconstruction</td>
<td></td>
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<tr>
<td>Yes</td>
<td>108 (50.5)</td>
<td>930 (86.0)</td>
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<tr>
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<td>106 (49.5)</td>
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<td>84 (39.2)</td>
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### Table

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<td>III</td>
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<td>207 (96.7)</td>
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<th>Locoregional recurrence</th>
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<tr>
<td>I</td>
<td>8 (3.7)</td>
<td>206 (96.3)</td>
</tr>
<tr>
<td>II</td>
<td>79 (7.3)</td>
<td>1002 (92.7)</td>
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<th>Distance metastasis</th>
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<tr>
<td>I</td>
<td>6 (2.8)</td>
<td>208 (97.2)</td>
</tr>
<tr>
<td>II</td>
<td>111 (10.3)</td>
<td>970 (89.7)</td>
</tr>
</tbody>
</table>

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<th>Survival</th>
<th>Yes</th>
<th>No</th>
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<tbody>
<tr>
<td>I</td>
<td>213 (99.5)</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>II</td>
<td>1014 (93.8)</td>
<td>67 (6.2)</td>
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</table>

**SLNB:** sentinel lymph node biopsy, **ALND:** axillary lymph node dissection.

**Conclusion:** The preliminary oncologic safety analysis from our institution showed that EABS is a safe procedure and results in low margin involved rate, and no increase of locoregional recurrence, distant metastasis or mortality compared with conventional breast surgery. However, this study might be biased due to its retrospective nature and possible selection bias. Ongoing case controlled comparison study will be performed to further consolidate the oncologic safety of endoscopic assisted breast surgery.
Radioactive seed and targeted axillary dissection: A feasibility study

Kerianne Boulva¹, Samuel Rodriguez-Qizilbash¹, Liliam Guilarte¹, Selma Lazizi¹, Andre Robidoux¹, Rami Younan¹ and Erica Patocskai¹. ¹Centre Hospitalier de l'Université de Montréal, Montreal, QC, Canada.

Targeted axillary dissection (TAD) with a radioactive seed is a new and promising technique to evaluate the axillary status in post-neoadjuvant chemotherapy (NACT) node-positive breast cancer patients. This study aims to evaluate the feasibility of TAD with a radioactive seed in a Canadian setting.

We conducted a retrospective observational study of a prospectively gathered database of patients having undergone TAD with a radioactive seed implanted in a proven metastatic axillary node, between 2015 and 2017 in our institution. An iodine-125 radioactive seed was implanted under ultrasound guidance by trained radiologists. Patients then underwent standard sentinel lymph node biopsy (SLNB) using technicium-99 and blue dye, as well as selective removal of the node containing the radioactive seed. Data was gathered from electronic medical records and chart review.

Nine patients with a median age of 54 underwent TAD for unilateral breast cancer post-NACT between 2015 and 2017, three of which were “triple-negative”. A median of 3 lymph nodes were removed, including that which contained the radioactive seed. Postoperative pathological evaluation showed 4 patients with positive nodes and 5 pathologic complete responses in the axilla. In all patients, the seed had been accurately positioned in a positive node (no false negatives). The seed also located a positive retropectoral node that would otherwise have been missed using standard dual tracer SLNB. No complications due to the use of the radioactive seed were encountered. All implanted seeds were identified and retrieved within the pathology specimen.

In our experience, TAD with a radioactive seed combined to dual tracer SLNB is an accurate and safe method of evaluating the axillary lymph node status in post-NACT node positive breast cancer patients. Further studies with larger cohorts are warranted to ensure continued feasibility and reproducibility of our positive results.
Title: Breast tumor location in BRCA mutation carriers and implications for prevention

Ava Hosseini¹, Laura J Esserman¹, Anne M Wallace², Alfred Au¹ and Rita A Mukhtar². ¹University of California, San Francisco, San Francisco, CA and ²University of California, San Diego, San Diego, CA.

Body: Introduction:
Close to 65% of BRCA mutation carriers do not choose prophylactic mastectomy, despite their high breast cancer risk. Breast reduction mammoplasty is a surgical technique shown to reduce breast cancer risk and can be modified to target specific areas of the breast. We wondered if a majority of tumors in BRCA mutation carriers would be confined to one quadrant, allowing for the use of targeted cosmetic mammoplasty as a novel method of risk reduction.

Methods:
We reviewed imaging reports on 103 consecutive patients with BRCA mutations and invasive breast cancer, and categorized tumor location by quadrant. Tumors spanning >1 quadrant were classified as being in both. Bilateral cancers were counted separately. Categorical variables were compared with the chi-squared test.

Results:
Mean age at breast cancer diagnosis was 44 years with mean tumor size of 2.2 cm (0.1-7cm). 92% of tumors were invasive ductal carcinoma, 46% were hormone receptor positive, 10% Her2 positive, and 44% triple negative. 70% of the tumors were unicentric. Tumors were significantly more likely to be in the upper outer quadrant whether or not multicentric tumors were included in the analysis (p<0.00001). Her2 positive tumors were more likely to be multicentric than other subtypes (p=0.021).

Conclusions:
More than half of breast cancers in BRCA mutation carriers form in the upper outer quadrant, suggesting that removing this quadrant through breast reduction mammoplasty could significantly reduce breast cancer risk. For women who are not ready for prophylactic mastectomy, this data supports an intermediate risk reduction step instead of only offering surveillance.
2017 San Antonio Breast Cancer Symposium

Publication Number: P5-22-23

Title: Electrochemotherapy: A new local therapy for cutaneous metastases of breast cancer

Martine Berliere¹, Elsa Raguzzi¹, Marion Bernard¹, Amandine Gerday¹, Maude Coyette¹, Philippe Piette¹, François Duhoux¹ and Benoît Lengele¹. ¹King Albert II, Cancer Institute, Cliniques Universitaires St Luc, Brussels, Belgium.

Body: Background: Electrochemotherapy (ECT) is a new local therapy combining the administration of an intravenous chemotherapy (bleomycin) followed by the direct application of electric pulses by an electrode to the treated areas. Electric pulses transiently permeabilize the membrane of the tumor cell (electroporation), improving intracellular diffusion of a poorly permeant chemotherapeutic agent, thereby increasing its cytotoxicity and decreasing systemic toxicity.

ECT can be offered to patients with cutaneous or subcutaneous metastases of breast cancer who are no longer suitable for extensive surgery or radiotherapy (e.g. due to past irradiation of the breast or chest wall).

ECT’s applications are palliative. It is delivered with the aim of local control of the tumor, relief of symptoms associated with cutaneous recurrence (ulceration, bleeding, pain), and improving patients’ quality of life.

The aim of this study is to demonstrate that ECT has a high efficacy and a low toxicity profile making it an interesting alternative to conventional therapies.

Material and methods: Our study is a retrospective study (approved by our local ethics committee) which included 8 patients between June 2013 and June 2016. The patients had histologically proven cutaneous and or subcutaneous metastases of their breast cancer, without any sign of lymphangitis. All the tumors expressed ER and/or PgR and 2 of them were HER2 positive tumors. The patients received a single or multiple courses of ECT; the outcomes were clinical response, toxicity, local and distant recurrence. The two patients whose tumors expressed HER2, also had synchronous contralateral axillary nodal metastases. They underwent axillary dissection performed during ECT course. Among the six other patients, no other metastases than cutaneous lesions were observed.

Results: The 8 included patients had already received chest wall irradiation for the treatment of their primary breast cancer. A total of 37 nodules were treated (mean: 4.5 per patient), whose mean size was 17mm (10-34mm). Six patients (75%) had a complete response after a single course of ECT. Two patients exhibited a partial response and underwent a second course of ECT at 6 months. The two patients with Her2 positive tumors received taxane-based chemotherapy and anti HER2 agents. All the patients were also given endocrine therapy. After a median follow up of 26 months (11-47 months), no patient had a local cutaneous recurrence. Overall survival rate was 75%: 2 patients died following progression of systemic disease (pulmonary and hepatic metastases).

No complications due to toxicity were observed.

Discussion and conclusion: In selected patients with cutaneous and subcutaneous metastases of breast cancer with no sign of lymphangitis, ECT offers a very good rate of clinical response and a durable control of metastases. This innovative technique has the advantage of being minimally invasive and well tolerated.
Title: Clinical and biological characterization of male breast cancer (BC) EORTC 10085/TBCRC 029/BOOG 2013-02/BIG 2-07: Baseline results from the prospective registry

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Body: BACKGROUND: Through the International Male Breast Cancer Program, a prospective registry for male BC was created with the goals of evaluating 1) the clinical and biological features of this disease and 2) assessing feasibility of a prospective therapeutic clinical trial.

METHODS: All men, with any stage histologically proven invasive breast cancer, age ³ 18 years, and newly presenting at the participating institutions (within 3 months prior) were eligible. Patients were enrolled for 30 months after activation of the first center, through February 2017. Per the study design, if <100 men enrolled, the study would be considered a failure and therapeutic trials would not be pursued through this network. Epidemiologic data, staging, pathologic features, and BRCA status were collected. Treatment and outcome data collection is ongoing. Optional collection of FFPE tumor samples, blood, and QOL were performed in the US, the Netherlands, and Latin America. Clinical database lock for this report was May 30, 2017. We currently report patient and disease characteristics and will update with patterns of treatment for the presentation. Outcomes and biological samples will be analyzed in the future.

RESULTS: 557 patients were enrolled: 75% in Europe, 20% in United States, 5% in other countries. 6.3% of patients had missing forms. Median age was 67 years (range 26-92). 93% were diagnosed 2010-2017. Among patients with complete data, 79% presented with a breast mass. 88% were M0 and 12% M1. Among M0 patients: 47%, 39%, 2%, and 11% had T1, T2, T3, and T4 disease respectively; 52% were N0. Overall, 98% had ER+ disease and 11% had HER2+ cancer. 14% had grade 1, 56% had grade 2, and 30% had grade 3 tumors. Among 112 men who underwent BRCA1 testing, 1 was positive. Among 118 men who had BRCA2 testing, 18 (15%) were positive. 21% of men had prior or concurrent malignancies, with the following most common sites: prostate, non-melanoma skin, colorectal, and melanoma. The prevalence of previously identified possible risk factors for male breast cancer were: overweight/obesity (72%), former/current smoker (51%), current alcohol ³1 drink daily (41%), family history of breast cancer (35%), gynecomastia (16%), history radiation exposure (8%), use of anti-androgens (1%), and use of estrogens (1%).

CONCLUSION: Through an international collaborative effort, we were able to prospectively accrue 557 patients to a male breast cancer registry. These results demonstrate feasibility of pursuing a therapeutic clinical trial in men with breast cancer. In addition, this study shows the relatively low uptake of BRCA testing, high rates of concurrent/prior malignancy, and the rates of potentially modifiable risk factors in this patient population.

Funding from Breast Cancer Research Foundation, Susan G. Komen, Dutch Pink Ribbon Foundation, Swedish Breast Cancer
Association (BRO) and EBCC Council.
Body: Introduction: Male BC is a rare disease (dx) for which management is extrapolated from trials in female BC. Comprehensive prospective data about QoL in men with BC could inform treatment. The international Male BC Consortium conducted a prospective registry of male BC patients of all stages who newly presented to a participating center between October 2013 and February 2017. A QoL substudy was conducted as part of this registry at most participating sites.

Methods: Informed consent for participation in the QoL substudy was requested from new enrollees. Those who consented were asked to complete a survey including the EORTC QLQ-C30 and BR23 (breast cancer specific module), adapted by replacing female-specific items with male-specific sexual activity/function items from the prostate module (PR25). Outcomes were scored according to standard EORTC QLQ procedures on a 0-100 scale (with higher scores on QoL/functioning scales representing better QoL and functioning, and higher scores on symptom scales representing worse symptoms). Forms were analyzed centrally by EORTC. In order to compare to female BC, we used reference data from 2782 mixed age (62% under age 60) women with BC (of whom 1,147 had recurrent or metastatic dx, and 464 had stage 1-2 dx) reported in the EORTC QLQ-C30 Reference Values manual (2008).

Results: A total of 557 men were enrolled in EORTC10085, 445 at sites participating in the QoL substudy. Consent forms were received from 422/445 (95%) for the substudy. Baseline survey (required to be completed within 30 days of enrollment) compliance was 85% (359/422). Median age at diagnosis was 67 years. There were 111 men (45%) with node-positive M0/MX dx and 27 men (8%) with M1 dx. Their median global health status score at baseline was 75 (IQR 67-83), higher than that documented historically in female BC (67, with IQR 50-83, in both the 2782 women with mixed stage and the subgroup of 464 with stage 1-2 tumors). The participating men's median social functioning score was 100 (IQR 67-100), also higher than the 83.3 (IQR 67-100) reported in mixed stage female BC patients, though no different than the 100 (IQR 67-100) found in women with stage 1-2 dx. Men's most commonly reported symptoms included fatigue (median score 13.9, IQR 0-33), insomnia (median score 0, IQR 0-33), and pain (median score 0, IQR 0-33), for which women's median scores were 33 (IQR 11-44), 33 (IQR 0-33), and 17 (IQR 0-50) with mixed stage dx, and 22 (IQR 0-33), 33 (IQR 0-33), and 17 (IQR 0-33) with stage 1-2 dx. Men's median sexual activity score was 33.3 (IQR 0-50), with less sexual activity reported by older patients and men with M1 dx. In those who were sexually active, median sexual function score was 83 (IQR 75-92), with no difference by age or stage.

Conclusions: QoL and symptom burden in male BC patients appears no worse (and possibly better) than that in female patients. Future analyses of 1- and 5-year surveys from this study will assess the impact of specific treatments on changes in symptoms and QoL over time. These data will be useful in future efforts to tailor treatments and target interventions for male BC.

2017 San Antonio Breast Cancer Symposium

Publication Number: P5-23-03

Title: Gene expression profiling in male breast cancer

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Body: Background:
Tumor gene expression profiling tests are widely used to quantify risk of recurrence of breast cancer and guide systemic therapy in early stage breast cancer. These assays have not been well validated in a male cohort. The purpose of this study was to determine the current rates of genomic testing in male breast cancer (MBC), the distribution of risk of recurrence scores (RRS) in early stage MBC, and the effect of RRS on systemic treatment patterns.

Methods:
The National Cancer Database was queried and found to include 6,227 cases of pathologic T1/T2 and N0/N1 MBC from 2008-2014 with known genomic testing status. Of the 1478 (23.74%) male patients who had gene expression profiling performed, variables of interest included age at diagnosis, stage, estrogen receptor status (ER), RRS, and treatment. We compared the treatment patterns of the 1,343 patients who had RRS available and the 4,527 patients who were not tested. A similar analysis was performed in female breast cancer (FBC) patients to serve as a point of reference. Statistical analysis included multivariate logistic regression and Pearson's chi-square test.

Results:
Of the 1,478 (23.74%) cases of MBC who had gene expression profiling, the most significant variables included: younger age, non-Black race, diagnosis after 2010, tumor Grade II, Estrogen Receptor (ER) positivity, and N0 or N1mi disease. Of those who had results, the distribution of RRS was 59.3% low, 27.4% intermediate, and 13.3% high. A similar distribution was found in 154,705 women who were tested during the same study period. Risk scores in men were significantly associated with tumor grade and size, but not nodal status. 83.4% of men with a low RRS were treated with hormone therapy alone, with an adjusted odds ratio (AOR) of 7.18 (CI 5.78-8.91, p<0.001). Also, 61.8% of men with a high RRS received combination chemotherapy and hormone therapy, with an AOR of 5.16 (CI 3.60-7.40, p<0.001).

Conclusion:
Although there is limited literature supporting the use of genomic assays in MBC, our study found similar rates of testing in men and women with early stage breast cancer. Treatment patterns varied significantly based on risk stratification, implying that physicians are using gene profiling assays to help guide treatment in MBC. Understanding the role for genomic profiling in MBC is particularly important as these results will be incorporated into the new AJCC 8th edition staging system. Long term follow up is needed to determine whether these tests accurately predict prognosis and recurrence in a male cohort.
**Title:** Men with advanced breast cancer (BC): Initial phase (Ph) 2 clinical activity of seviteronel, a selective CYP17-lyase and androgen receptor (AR) inhibitor

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**Body: Background:**
Seviteronel (Sevi), an oral selective CYP17-lyase and AR inhibitor that blocks testosterone and estradiol production whilst competitively antagonizing the AR, is in Ph 2 clinical development for the treatment of advanced prostate and breast cancers (BC). Male BC is a high unmet need. Men with BC have a high rate of advanced disease at the time of diagnosis and disease-related deaths but no approved therapies. A majority of male BC tumors are AR+ (≥85%; Sas-Korczynska et al. Pol J Pathol. 2015; 66:347-52) suggesting a role for AR-directed therapies. The primary objective of this ongoing Ph 2 study (NCT02580448) is to estimate the activity of once daily Sevi in men with BC as measured by clinical benefit rate (CBR; proportion of pts with stable disease/partial response/complete response) at 16 weeks (wks).

**Methods:**
Male patients (pts) with ER+/HER2-normal locally advanced or metastatic BC following progression of at least 1 prior line of endocrine therapy were enrolled. Pts were ECOG 0 or 1 and undergoing gonadal suppression at the time of study entry. Evaluable (Eval) pts received at least 1 dose of Sevi, had 1 post-baseline scan, and were either discontinued or were on study at wk 16. Sevi was administered at 600 mg oral daily. Scans were performed every 8 wks. A Simon's 2-stage design was employed to determine activity (≥1 of 7 Eval pts with CBR16 allowed for accrual to Stage 2; total Ph 2 enrollment target of 18 Eval pts).

**Results:**
As of 01 June 2017, 7 men with BC were enrolled. Median age was 58y [53, 70] (median [range]) and 71% were ECOG = 1. 71% had visceral metastases; 14% with brain, 43% with liver and 57% with lung involvement. Median lines of prior therapies for BC in any setting was 9 [5, 22]. All pts enrolled had at least one line of prior therapy for advanced disease and 71% had ≥2 prior lines; these included hormonal therapy (14%), chemotherapy (14%) and hormonal + chemotherapy (43%). Median Sevi treatment duration was 58d [41, 185] and one pt is ongoing. One pt met CBR16, allowing for accrual to Stage 2. All discontinuations were for disease progression (n=6). The most common adverse events (≥2 pts) were fatigue (71%), back pain (29%), constipation (29%), decreased appetite (29%), dizziness (29%), hot flushes (29%), insomnia (29%) and nausea (29%); all Grade 1/2 except for one case of Grade 3 nausea. Updated duration of treatment and CBR data will be presented at the time of presentation.

**Conclusions:**
Seviteronel is active in male BC and was well tolerated with no discontinuations due to AEs. Stage 1 criteria for Sevi activity was met and full Ph 2 enrollment is ongoing for men with BC. In comparison to women with ER+ BC treated with Sevi in Ph 2 (Gucalp et al, ASCO 2017), men had more advanced disease (e.g., visceral disease and prior treatments for metastatic disease). Sevi may provide a novel treatment option for ER+ BC in heavily pre-treated men with high disease burden.
Title: Prognostic value of PD-L1 expression in male breast cancer

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Body: Background
Growing evidence have shown promise for targeting programmed death-1 (PD-1)/programmed death-ligand 1 (PD-L1) signaling in several tumors. In female breast cancer, PD-L1 expression has been correlated with poor clinical outcome. However, whether PD-L1 has any indication for prognosis in male breast cancer (MBC) patients remains unknown. The purpose of this study was to examine the expression levels of PD-L1 in MBC and to identify the relationship between PD-L1 expression and patient survival.

Methods
We retrospectively identified 110 male breast cancer patients diagnosed between 2000 and 2013 at Salah Azaïz Cancer Institute. PD-L1 expression was evaluated by immunohistochemistry (IHC) using CD274 antibody. Specimens were scored as IHC low or high, when < 5% or ≥ 5% of cells were PD-L1 positive, respectively. The association between expression of PD-L1 and survival was investigated using Kaplan-Meier survival and COX proportional hazard regression analyses.

Results
Median follow up was 12.5 months [range 1-132 months]. High expression of PD-L1 was observed in 64.5% of MBC samples (71/110). PD-L1 expression was significantly associated with advanced clinical stage (p=0.012), higher histological grade (p=0.014), higher Ki67 expression (p=0.023) and hormone-receptor negative status (p=0.024). Patients with high PD-L1 expression had significantly shorter overall survival (OS) than patients with low expression (p=0.002, hazard ratio (HR) =5 [2.624–10.642]). Multivariate analysis identified PD-L1 as an independent prognostic factor for OS (p<0.001, HR = 0.775 [0.680–0.870]).

Conclusion
Our results indicate that high PD-L1 expression may be a prognostic indicator for reduced OS. Thus, PD-L1 expression is a promising novel biomarker with prognostic significance in MBC and may suggest a potential therapeutic target of anti-PD-L1 antibody therapy in MBC patients.
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Publication Number: P5-23-06

Title: Metastatic pattern in stage IV male breast cancer at initial diagnosis: A population-based study

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Body: Background: Male breast cancer (MaBC) is a relatively uncommon disease, representing less than 1% of all breast cancers. Although men tend to present at more advanced stages, the prognostic influence of metastatic pattern (MP), sites of metastases and factors associated with specific organ involvement are unknown. The primary aim of this study was to analyze the influence of MP compared with other biologic and clinical factors in the survival of patients (pts) with stage IV breast cancer at initial diagnosis (BCID). Secondary aims were to describe sites of metastases and evaluate factors associated with specific sites of metastatic spread.

Methods: We evaluated men with microscopically confirmed stage IV BCID with known metastatic sites, reported to the Surveillance, Epidemiology and End Results (SEER) 18 registries program from 2010 to 2014. Pts with other primary tumor either before or after breast cancer were excluded. MP was categorized as bone only, visceral (lung, liver or brain), bone and visceral and other. Pt characteristics were compared between MP. Univariate and multivariate analyses determined the effects of each variable on overall survival (OS). Logistic regression examined factors associated with specific sites of metastases.

Results: We included 136 pts. Median age was 63 years (range 28-91). At diagnosis, bone only metastases represented 31.6% of pts, visceral 16.2%, bone and visceral 38.2% and other 14%. Median OS for the entire cohort was 33 months (95% CI 19 months – not reached). Bone was the most common site of metastases (69.9%), followed by lung (44.9%), liver (14%) and brain (8.1%). OS rate at 3 years by MP was: bone only 46.05%, visceral 53.57%, bone and visceral 37.23% and other 70.56% (p=0.41). There were no significant differences in pt or tumor characteristics between MP. However, in adjusted logistic regression, triple negative (TN) and ER/PR+/HER2+ tumors had higher odds of brain metastases than ER/PR+/HER2- (all p<0.05). Also, ER/PR+/HER2+ tumors had higher odds of liver metastases (p=0.027). Univariate analysis showed that older age (HR 1.7; p=0.03), no surgery (HR2.5; p=0.005), TN tumors (HR 5.6; p<0.001) and pts with brain metastases (HR 4.2; p<0.001) had worse prognosis. In multivariate analysis, TN subtype (HR 4.2; p=0.001) and pts with brain metastases (HR 3.44; p=0.012) had significantly shorter OS.

Conclusions: To our knowledge, this is the first study of MP in MaBC. The cohort had an acceptable median OS which did not differ significantly according to MP. Although brain metastases were less common, it is important to recognize their worse prognosis. Independent predictors of OS included tumor subtype and brain metastases. Tumor subtype had a clear influence on specific sites of metastases, particularly for brain and liver involvement.
Title: Patterns of disease progression in patients with local and metastatic breast cancer as evaluated by whole-body MRI

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Body: Background
Clinical evaluation of palpable breast or chest wall disease is important in the assessment of response to systemic therapy in patients with combined local and metastatic breast cancer. However, there is a lack of data describing the patterns of progressive disease (PD) in relation to local and metastatic sites in patients on systemic anti-cancer therapy (SACT).

Whole-body magnetic resonance imaging (WB-MRI) provides detailed information about the extent and distribution of local and metastatic disease in breast cancer. Recent published data has shown the superiority of WB-MRI over body CT scans at identifying sites of disease and therapy response.

This retrospective study is designed to analyse the patterns of PD on WB-MRI, in patients on first line SACT for combined local and metastatic breast cancer.

Methods
Patients with stage IV disease at first diagnosis, or those previously treated for early breast cancer with local (breast or chest wall) and metastatic recurrence were included. Patients were eligible for analysis if they had a baseline WB-MRI prior to starting first line SACT for metastatic disease, and subsequent WB-MRIs for response assessment up to the point of PD and a change in SACT.

Patient information and SACT data were collected from contemporaneous medical records. Data on sites of disease and sites of progression were collected from the original WB-MRI reports. All WB-MRI scans were performed using a published WB-MRI protocol.

Results
Thirty-one patients were suitable for analysis. Eighteen had metastatic disease at first presentation of breast cancer. Thirteen had both local and metastatic disease recurrence, 8 after previous mastectomy and 5 after previous breast-conserving surgery. Mean age of patients at metastatic diagnosis was 58.3 years (range 31 – 86 years). Fifteen patients received first-line chemotherapy, 10 with Her2-targeted therapies. Sixteen received first-line hormonal therapy.

None of the patients progressed first in local disease and/or regional nodes only. Seven patients (22.6%) had evidence of first PD in their loco-regional disease along with concurrent PD at metastatic sites. Twenty-four patients (77.4%) had evidence of first PD at metastatic sites without progression of loco-regional disease.

Of the 26 patients with bone metastases, 20 had PD of bone disease. Two patients without bone disease at baseline had new bone lesions evident at first PD.

Of the 8 patients with liver metastases, 4 had progression of liver disease. Five patients without liver disease at baseline had new liver lesions evident at first PD.

None of the patients had a change in SACT performed for clinical disease progression only.

Conclusions
PD identified by WB-MRI occurs most commonly at metastatic sites rather than at palpable sites of loco-regional disease. Therefore, evidence of ongoing clinical benefit of SACT at loco-regional sites of breast cancer on clinical examination may give false reassurance about the disease status at metastatic sites. Regular imaging reassessments are advised for patients undergoing SACT for loco-regional and metastatic disease.
Title: Spatial heterogeneity of response on whole-body MRI to first line hormonal therapy predicts progression-free survival in metastatic breast cancer

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Body: Background
Whole-body magnetic resonance imaging (WB-MRI) reliably identifies types of response to systemic therapy in metastatic breast cancer (MBC) through analysis of changes in water diffusivity, cellularity and cell variability.
We adapted a novel methodology that uses WB-MRI to capture spatial response heterogeneity data via the METastasis Response Assessment Diagnostic System (MET-RADS).
This study evaluates whether the extent of spatial heterogeneity seen at initial response assessment is predictive of progression-free survival (PFS) in patients on first line hormonal therapy for MBC.

Methods
Patients on first line hormonal therapy for MBC who had undergone baseline and on-treatment response assessment WB-MRI scans were identified. All scans were performed using a published WB-MRI protocol. Patients with disease progression at their first WB-MRI response assessment were excluded from further analysis.
Criteria for response assessment utilised the methodology described by MET-RADS. A Likert five-point Response Assessment Category (RAC) score (1=response highly likely; 2=response likely; 3=no change; 4=progression likely; 5=progression highly likely) was applied separately to 14 defined anatomic regions. Within each region, two RAC scores were recorded to reflect both the dominant and the next most common pattern of response behaviour. Data were therefore captured on inter- and intra-regional response heterogeneity.
A novel Response Heterogeneity Index (RHI) was calculated from the regional RAC scores. The RHI value summarised the overall response heterogeneity seen across all involved regions, with higher scores indicative of greater heterogeneity of response. Depth of overall response to treatment was defined as the mean RAC score for all involved regions, with lower scores indicative of a greater depth of treatment response. RHI and mean RAC score data were separated by median values into two groups for analysis.

Results
Thirty-three patients were suitable for analysis. Patients with higher levels of response heterogeneity (defined as RHI ≥4; n=16) had significantly shorter PFS than those with RHI <4 (n=17; median PFS: 12 vs 24 months; log rank test p=0.006).
Depth of initial response, as defined by mean RAC score, and PFS were unrelated (mean RAC ≤2.5 (n=17) vs mean RAC >2.5 (n=16); median PFS: 27 vs 23 months; log rank test p=0.699).
There was no correlation between RHI score and mean RAC score (r²=0.002).

Conclusions
Lower variability of response behaviour at first treatment assessment is predictive of longer treatment duration in patients receiving first line hormonal therapy for MBC.
The spatial heterogeneity of response evident on WB-MRI may represent the genetic heterogeneity and clonal evolution of MBC, which are key factors in the development of treatment resistance.
Objective: Screening mammography (SM) benefits are maximal in women who have a several years' life expectancy. Perceived life expectancy (PLE), however, can be compromised by older age, and/or poor health. Prior studies have shown low rates of SM in younger and/or healthier women, while women who are older and/or those presenting with multimorbidity (MM) continue to undergo screening. The resulting under/overuse of SM causes an imbalance in the use of finite resources that should be rectified as we prepare for population aging. In this study, we investigate the use of SM in women 50 years of age or older in the context of age, PLE, MM, and other social determinants of health.

Methods: This is a cross-sectional study using the nationally-representative 2012 U.S. Health and Retirement Study (n= 8934 women). In addition to demographics, we examined a broad range of variables on social determinants of health; as well as conditions constituting MM, including self-reported chronic conditions (e.g., heart disease), functional limitations (e.g., strength, and mobility limitations), and geriatric syndromes (e.g., low cognitive performance). We defined MM0-MM3 as gradients of MM, based on the occurrence/co-occurrence of chronic conditions, functional limitations, and/or geriatric syndromes. PLE was calculated based on respondent's age-specific assessment of the chance they would live another 10-15 years. The outcome variable was self-reported mammography in the past 2 years. We conducted logistic regression analysis to evaluate the independent and interactive association between each of PLE and MM relative to SM, adjusting for potential confounders.

Results: The median age was 65.7 years; 10.5% were Non-Hispanic Black (NHB), 7.8% were Hispanic, and 3.2% were Other Race. The percent of women with SM was 71.5% in the total population. The median assessment of PLE was a 50% chance of living another 10-15 years. 71.9% of women presented with chronic conditions, 36.8% with functional limitations, and 58.2 with geriatric syndromes. Adjusting for confounders, receipt of mammogram was positively associated with greater certainty of PLE (AOR for an increase of 10% –1.03 (1.01, 1.05)). Compared to those with no conditions (MM0), the co-occurrence of chronic conditions, functional limitations, and/or geriatric syndromes was also positively associated with SM (AOR MM1 – 1.48 (1.19, 1.84); AOR MM2 – 1.35 (1.09, 1.69); and AOR MM3 – 1.29 (1.02, 1.62)). In addition, PLE further strengthened the association between MM and SM. Although age was negatively associated with SM, this interacted with PLE such that the likelihood of having a mammogram was unrelated to age among women 100% certain they would live another 10-15 years.

Conclusion: Both multimorbidity (MM) and perceived life expectancy (PLE) are independently and interactively associated with increased screening mammography (SM), suggesting overuse. Indeed, even among women 75 years of age or older, when SM may be least beneficial, receipt of SM in the past two years was 59.6% in the presence of highest MM gradient, and 68.1% when they were at least 75% certain they will live 10-15 years. A more detailed examination of the basis for PLE is warranted to understand the context in which screening recommendation is made.
Title: Variables influencing HER2-positivity in breast cancer: Assessment and validation of a statistical model based on two multicenter noninterventional studies in Germany

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Body: Background:
HER2 testing in breast cancer (BC), routine for >10 years, allows selection of patients (pts) for HER2-targeted therapy; however, testing quality remains a concern. While guidelines recommend assessment of HER2-positivity rates as a quality indicator, the influence of patient- or tumor-related factors on variability was unknown until we identified the effect (in order of influence) of histologic grade, hormone receptor (HR) status, histologic subtype, age, and nodal status in a large, multicenter, observational study in Germany (NIU HER2 study; Rüschoff et al., Mod Pathol 2017). Based on these variables and the statistical model developed, potential issues with HER2 testing quality in local practice may be identified. We now report interim analyses from a multicenter study in Germany (EPI HER2 BC study; NCT02666261), where data from the NIU and EPI studies were compared and the validity of the NIU study model assessed.

Methods:
Routine HER2 test results and patient- and tumor-related data were collected from eligible pts with BC. Factors influencing HER2-positivity rates in the EPI study were compared with those identified in the NIU study. The predictive power of the NIU study model, fitted to EPI data, was determined and assessments performed using the variable coefficients and cutoff resulting from the NIU study analysis. Attempts were also made to improve the model.

Results:
Analyses included 15281 (NIU) and 6019 (EPI) invasive BC samples. The distribution of relevant variables, including HER2-positivity rate (NIU: 14.4%; EPI: 13.5%), was comparable. When the NIU study model was fitted to EPI study data, all five covariates identified in the NIU analyses had a significant effect on HER2-positivity (p<0.001); the order of influence for covariates differed between studies (EPI [in order of influence]: histologic grading, histologic subtype, HR status, nodal status, and age). The relationship between HER2-positivity rate and the combined influence of covariates, visualized with the NIU study prediction profiler, was reproduced with EPI study data. The NIU study statistical model, with variable coefficients and cut-point determined in the NIU study, was used to predict the HER2-positivity of samples in EPI; if their NIU model-estimated probability of positivity was >0.1407, the resulting sensitivity, specificity, and receiver operating characteristic (ROC) area under the curve (AUC) were 0.7032, 0.6622, and 0.7259, respectively. Thus, initial validation of the NIU study model with EPI data was successful. Semiquantitative estrogen and progesterone receptor expression data were available from EPI only; their inclusion as independent continuous, rather than categorical, variables improved the model (ROC AUC = 0.7533).

Conclusions:
The statistical modeling approach used to analyze data from the NIU study showed that patient- or tumor-related characteristics should be considered when assessing HER2 testing quality. Our present analysis validates and improves upon this statistical model and further highlights the need to assess HER2 testing quality in BC. Comparison of calculated vs actual positivity rates may help identify centers with potential HER2 testing quality issues.
**Title:** Independent evaluation of prognostic value of residual cancer burden (RCB) score on disease free and overall survival of breast cancer patients treated with neoadjuvant systemic treatment at a single institution

Marija Balic¹, Deborah Hannah Mueller¹, Robert Hammer², Melanie Gumpoldsberger¹, Christoph Suppan¹, Florian Posch¹, Herbert Stoeger¹, Nadia Dandachi¹, Kurt Prein³, Hubert Hauser² and Sigurd Lax³. ¹Comprehensive Cancer Center, Medical University Graz, Graz, Austria; ²Landeskrankenhaus Sued West Standort West, Brustzentrum, Graz, Austria and ³Pathology Institute, Landeskrankenhaus Sued West Standort West, Brustzentrum, Graz, Austria.

**Body:**

**Introduction:** Among the various approaches to measure response to neoadjuvant chemotherapy in breast cancer patients a significant relationship to overall (OS) and disease-free survival (DFS) has been recently demonstrated for the residual cancer burden (RCB) score. The calculated RCB- index provides additional prognostic information independently of TNM categories and stage, respectively[1]. The goal of our study was to validate the prognostic impact of the RCB score for OS and DFS on a cohort of patients selected for neoadjuvant treatment and treated either with chemotherapy with or without Her2 targeted therapy or endocrine therapy. We hypothesised that across all histological breast cancer subtypes, the RCB-score will show significant impact on OS and DFS.

**Methods:** All surgical specimens (n =212) were processed according to the same protocol in a single institution. The RCB score was calculated according to Symmans et al. using the MD Anderson website. Pathologic complete remission is designated RCB 0, residual disease is based on its extent categorized as RCB I-III. Additional information such as histological and molecular subtypes, therapy regimen, as well as follow up information were collected prospectively from paper based patient files. The impact of the RCB score on OS and DFS were estimated with Kaplan-Mayer curves were compared by using long rank test statistics.

**Results:** The RCB score showed a significant correlation to DFS (p=0.000006) and OS (p=0.000306) The correlation between molecular subtypes (Luminal A, luminal B, Her2 positive and triple negative) and neoadjuvant treatment is ongoing and will be presented at the meeting.

**Conclusion:** We were able to show a general prognostic value of the RCB-Score in a series of 212 patients with neoadjuvant treatment, without consideration of the histological and molecular subtypes and adjuvant treatment. We were further able to confirm the reproducibility of the RCB Score by a standardized pathological procedure at a single institution. Further independent evaluation of the histological and molecular subtypes as well as the therapy regimens is ongoing.

Title: Clinical validation of Videssa® breast in women over age 50 with BI-RADS® 3, 4, or 5 assessment

Meredith C Henderson¹, Michael Silver², Quynh Tran², Rao Mulpuri², Elias Letsios², David E Reese² and Judith Wolf². ¹Provista Diagnostics, Scottsdale, AZ and ²Provista Diagnostics, New York, NY.

Body: Introduction:
The ability to detect Breast Cancer (BC), including ductal carcinoma in situ (DCIS), in a precise manner remains a challenge. False-positive imaging results can lead to potentially avoidable breast biopsies; there is an unmet clinical need for a noninvasive diagnostic assay to help determine whether a breast biopsy is warranted. Combinatorial biomarker panels, in conjunction with imaging, were developed to accurately detect BC independently using a serum-based approach (Videssa® Breast). Prior studies demonstrated effectiveness in women under the age of 50; this study tests performance in women over age 50.

Methods
The Provista-002 trial enrolled 1,005 women across 2 cohorts in a national, multi-center, blinded, and randomized manner. Serum was collected after a BI-RADS 3, 4 or 5 assessment (as assessed by any imaging modality including mammography, ultrasound, 3-D Tomosynthesis, or MRI) and blood was drawn prior to biopsy. A total of n=663 subjects were randomized into training (n=469) and validation (n=194) groups. Serum was evaluated for 16 Serum Protein Biomarkers (SPB) and 48 Tumor Associated Autoantibodies (TAAb). These measurements were combined with participant clinical data to train and validate a model that can reliably determine the presence or absence of BC in this population. The final model was optimized for a biopsy rule-out indication (high sensitivity and negative predictive value- NPV).

Results:
Logistic Boost models were built using a SMOTE approach to compensate for low cancer prevalence and clinical factors were assessed to boost clinical sensitivity. The resulting model score distributions differed greatly between each BI-RADS® category. To avoid bias introduced by BI-RADS®, separate cutpoints were selected for BI-RADS® 3 subjects and for BI-RADS® 4 and 5 subjects. Cutpoints were optimized for maximum sensitivity and NPV. The final model resulted in 95% sensitivity and 97% NPV in the blinded validation cohort. As such, a negative test result would be false-negative in only 3% of cases. Of the n=432 BI-RADS 3 or 4 subjects who underwent biopsy, a total of 89 subjects (21%, or approximately 1 in 5) could have been spared biopsy had the Videssa® Breast results been incorporated in the clinical workflow.

Conclusions:
Videssa® Breast was developed to accurately detect BC and reduce false positives in women with clinically suspicious findings, the population for which the majority of image-based false positives occur. When combined with imaging, Videssa® Breast could ultimately reduce the number of unnecessary biopsies, thereby increasing the accuracy of BC detection in post-menopausal women.

Trial Registration:
ClinicalTrials.gov, NCT01839045 and NCT02078570
Central nervous system miliary metastasis in breast cancer patients

Sami I Bashour1, Nuhad K Ibrahim1, Donald F Schomer1, Rivka R Colen1, Raymond Sawaya1, Dima Suki1, Ganesh Rao1, Rashmi K Murthy1, Stacy L Moulder1, Yehia Abugabal1, Kenneth R Hess1 and Gregory N Fuller1. 1The University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Background: Little is known regarding central nervous system (CNS) miliary metastasis (MiM), which was first described as "carcinomatous encephalitis" by Madow and Alpers in 1951. The majority of reported cases arise from primary lung and gastrointestinal adenocarcinomas, with occasional melanoma primaries and one reported case in breast cancer. Moreover, clinicopathologic correlates, disease outcomes and prognostic factors in these patients are poorly understood. Although identified most frequently on neuroimaging, radiographic criteria to objectively diagnose MiM do not exist. In this analysis of patients with brain metastasis from primary breast cancer, we propose objective, stringent radiographic criteria for CNS MiM diagnosis and identify clinicopathologic factors contributing to disease outcomes.

Methods: Using a prospectively maintained electronic database, 1,002 female patients diagnosed with brain metastasis from primary breast cancer between 2000 and 2015 were identified. Only patients with neuroimaging available for direct review (CT or MRI) were included. Our radiographic criteria for MiM diagnosis were: 1) ≥20 metastatic lesions per image slice on ≥2 noncontiguous image slices by MRI, or 2) ≥10 lesions per image slice on ≥2 noncontiguous image slices by CT, and 3) MiM lesions were required to be present bilaterally and in both the supra- and infratentorial compartments. These criteria were established upon direct review of all neuroimaging by a neuroradiologist. Number and anatomic distribution of metastatic lesions were the patterns best observed to identify cases of CNS MiM on case review; lesion size was not a reliable pattern for MiM identification. Log rank tests were used for statistical analyses.

Results: Using stringent criteria, 486 patients were included in this analysis. Twenty patients with MiM were identified (4.1%). Ten patients were diagnosed with MiM at initial brain metastasis presentation; 10 developed MiM after known brain metastasis. Biomarker based subtype distribution was as follows: HR-/HER2- (TNBC) (n=8), HR+/HER2+ (n=3), HR+/HER2- (n=4), HR-/HER2+ (n=4), unknown (n=1).

Table 1: Disease Outcomes Based on Biomarker Subtype

<table>
<thead>
<tr>
<th>Biomarker Subtype</th>
<th>Median Time to MiM (months) (p=0.104)</th>
<th>Median Survival after MiM (months) (p=0.008)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNBC (n=8)</td>
<td>32.3 (12.1-132.5)</td>
<td>1.8 (0.5-4.0)</td>
</tr>
<tr>
<td>HR+/HER2+ (n=3)</td>
<td>44.2 (33.2-71.5)</td>
<td>10.8 (10.2-13.3)</td>
</tr>
<tr>
<td>HR+/HER2- (n=4)</td>
<td>110.2 (23.0-156.0)</td>
<td>4.8 (0.8-9.8)</td>
</tr>
<tr>
<td>HR-/HER2+ (n=4)</td>
<td>27.1 (3.7-39.4)</td>
<td>4.0 (1.8-5.0)</td>
</tr>
<tr>
<td>All* (n=20)</td>
<td>37.4 (3.7-156.0)</td>
<td>3.7 (0.4-12.3)</td>
</tr>
</tbody>
</table>

Key: BM: Brain metastasis; * Includes 1 patient with unknown subtype.

Conclusions: Reports of MiM consist overwhelmingly of lung and gastrointestinal adenocarcinoma primaries. This retrospective, observational study is the first to establish that CNS MiM occurs in breast cancer with an incidence of roughly 4%. Review of an additional 1,600 patient charts is underway, but this preliminary study is the first to identify clinicopathologic correlates and determine disease outcomes in patients with MiM; it is also the first to propose stringent radiographic criteria for the diagnosis of CNS MiM, and further updated data will be presented at the meeting.
Risk of needle-track seeding with serial ultrasound guided biopsies in triple negative breast cancer

Clinton Yam\textsuperscript{1}, Lumarie Santiago\textsuperscript{1}, Rosalind P Candelaria\textsuperscript{1}, Beatriz E Adrada\textsuperscript{1}, Gaiane M Rauch\textsuperscript{1}, Kenneth R Hess\textsuperscript{1}, Jennifer K Litton\textsuperscript{1}, Helen Piwnica-Worms\textsuperscript{1}, Elizabeth A Mittendorf\textsuperscript{1}, Naoto T Ueno\textsuperscript{1}, Bora Lim\textsuperscript{1}, Rashmi K Murthy\textsuperscript{1}, Senthil Damodaran\textsuperscript{1}, Thorunn Helgason\textsuperscript{1}, Lei Huo\textsuperscript{1}, Alastair M Thompson\textsuperscript{1}, Michael Z Gilcrease\textsuperscript{1}, W Fraser Symmans\textsuperscript{1}, Stacy L Moulder\textsuperscript{1} and Wei Yang\textsuperscript{1}.\textsuperscript{1}The University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Background: Image-guided percutaneous needle biopsy of the breast is a common procedure. In breast cancer patients (pts) undergoing core biopsies and surgical resection on the same day, the rate of tumor cell displacement along the needle track has been reported to be up to 50%. However, the clinical significance of this finding in triple negative breast cancer (TNBC) patients (pts) undergoing serial biopsies while receiving neoadjuvant chemotherapy (NACT) is unknown. Here we report the incidence of needle-track seeding (NTS) in a cohort of TNBC pts enrolled on a molecular triaging protocol involving serial biopsies of the index breast lesion.

Methods: We reviewed the clinical records of 144 consecutive TNBC pts enrolled on a molecular triaging protocol at MD Anderson Cancer Center. Per protocol, all pts underwent a pre-treatment research biopsy and were initiated on anthracycline based NACT (AC). Pts with inadequate response to front-line NACT were encouraged to undergo additional biopsies of the index breast lesion prior to switching therapies. Serial breast ultrasound (US) was performed to monitor therapeutic response and incidental evidence of needle-track seeding noted on US was documented.

Results: Clinicopathological characteristics of the pts are summarized in Table 1. 89% (128/144) of pts had a diagnostic breast biopsy done at another center prior to presenting at MDACC. To date, we have performed 209 US guided biopsies of index breast lesions in 144 pts. 92% (193/209) of these biopsies were done mainly for research purposes. 1.4% (2/144) of pts were found to have evidence of NTS on follow up US. The first pt had a T1N0 (1.9cm), grade 3, invasive ductal carcinoma (IDC) at diagnosis. She underwent a diagnostic biopsy followed by a research biopsy before initiating AC. She was found to have NTS as well as progression of disease (PD) on follow up US after 2 cycles of AC. The second pt had a T2N0 (3cm), grade 3 IDC at diagnosis. She underwent a diagnostic biopsy at another center, followed by a research biopsy before initiating AC. Like the first pt, she was found to have NTS and PD on follow up US after 2 cycles of AC. Both pts are currently on neoadjuvant clinical trials of novel agents.

Conclusion: The rate of NTS detected on US in TNBC pts undergoing serial biopsies of index breast lesions while receiving NACT is low and further studies are needed to determine the impact of serial biopsies on long term outcomes in TNBC.

Table 1: Patient Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N=144</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age - Median (years, interquartile range)</td>
<td>55 (46-62)</td>
</tr>
<tr>
<td>Tumor Size</td>
<td></td>
</tr>
<tr>
<td>Mean (cm, standard deviation)</td>
<td>3.4 (2.2)</td>
</tr>
<tr>
<td>T1 – n(%)</td>
<td>35 (24)</td>
</tr>
<tr>
<td>T2 – n(%)</td>
<td>89 (62)</td>
</tr>
<tr>
<td>T3 – n(%)</td>
<td>19 (13)</td>
</tr>
<tr>
<td>T4 – n(%)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Clinical Nodal Status</td>
<td></td>
</tr>
<tr>
<td>Negative – n(%)</td>
<td>74 (51)</td>
</tr>
<tr>
<td>Positive – n(%)</td>
<td>70 (49)</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
</tr>
<tr>
<td>Category</td>
<td>Count (%)</td>
</tr>
<tr>
<td>-------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>1 – n(%)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>2 – n(%)</td>
<td>17 (12)</td>
</tr>
<tr>
<td>3 – n(%)</td>
<td>124 (86)</td>
</tr>
<tr>
<td>Unknown – n(%)</td>
<td>2 (1)</td>
</tr>
</tbody>
</table>

**Histologic Subtype**

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Count (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invasive ductal carcinoma – n(%)</td>
<td>121 (84)</td>
</tr>
<tr>
<td>Invasive lobular carcinoma – n(%)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Mixed ductal and lobular carcinoma – n(%)</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Metaplastic carcinoma – n(%)</td>
<td>13 (9)</td>
</tr>
<tr>
<td>Not specified – n(%)</td>
<td>5 (3)</td>
</tr>
</tbody>
</table>

**Laterality**

<table>
<thead>
<tr>
<th>Laterality</th>
<th>Count (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right – n(%)</td>
<td>72 (50)</td>
</tr>
<tr>
<td>Left – n(%)</td>
<td>72 (50)</td>
</tr>
</tbody>
</table>
2017 San Antonio Breast Cancer Symposium

Publication Number: P6-03-06

Title: Assessing occult brain metastasis with CT scan or MRI in HER2-positive breast cancer patients at initial diagnosis

Catherine Delbaldo¹, Thomas Sarrade², Bertrand Brieau³, Bertrand Billemont⁴, Jennifer Denis⁵, Isabelle Cojean-Zelek⁶, Carole Bornier⁷ and Etienne Vincens⁸. ¹Service d’Oncologie, Hôpital Diaconesse-Croix Saint- Simon, Paris; ²Service d’Oncologie Curie-René Huguenin, Saint Cloud; ³Service d'Oncologie, Hôpital Cochin, Paris; ⁴Service d'Oncologie Médicale, Centre Hospitalier, Noumea, Nouvelle Calédonie; ⁵Service d'Oncologie, Hôpital Diaconesse-Croix Saint- Simon, Paris; ⁶Service d'Oncologie, Centre Inter Communal, Créteil; ⁷Service de Radiologie Hôpital Diaconesse-Croix Saint- Simon, Paris and ⁸Service de Gynécologie Hôpital Diaconesse-Croix Saint- Simon, Paris.

Body: Patients (pts) with HER2-positive breast cancer (BC) have a high risk of developing brain metastases (BM) that might be due to longer survival, low diffusion of Trastuzumab (T) in the CNS or an unknown incidence of occult BM at diagnosis. In a series of metastatic BC, occult metastasis was found in 15% of the patients and Her2 over expression was an independent risk factor.¹ In a series of early BC treated with T the cumulative incidence of BM at 12 and 24 months was 0.6 and 2% respectively.² Time to BM was 13.7 months in retrospective study.³ Systematic cranial magnetic resonance imaging (MRI) in the staging of HER2-positive BC pts detected BM in 22% of asymptomatic, metastatic pts versus 3.6% in asymptomatic, non-metastatic pts.⁴ Early detection of BM decreases the cerebral death rate, but do not prolong survival in metastatic pts.⁴ Therefore brain imaging is not recommended at diagnosis. However, retrospective trials assessing the incidence of BM in HER2-positive BC were mainly performed during follow up and not at the initial diagnosis.

Pts treated for HER2-positive locally BC with adjuvant sequential chemotherapy including anthracycline and taxane associated with T, classified according to classical prognosis factor as histological subtype, TNM, SBR and hormonal receptor (HR), were systematically assessed for occult BM at initial diagnosis with CT scan or MRI. Retrospectively analyzed for the occurrence of BM was performed. Disease free metastatic survival (DFMS) and overall survival (OS) was estimated by Kaplan Meier estimation. Between March 2006 and July 2013 84 pts were included. The median follow up is 4.7 years. The characteristics pts were median age 57 years [30-86], ductal carcinoma 67 pts (93%), lobular carcinoma 5 pts (7%), pT1 to 4 respectively 38 (48%), 28 (36%), 3 (4%), 9 pts (12%), pN 0 to 3 respectively 44 (54%), 27 (33%), 5 (6%), 5 pts (6%), SBR 1 to 3 respectively 1 (1%), 35 (43%), 43 pts (54%), RO positive 47 pts (59%), RP positive 27 pts (63%). Thirty eight pts (45%) had brain imaging at diagnosis or within 5 months of diagnosis. Only one pt with a pT4, pN2, ductal carcinoma, RO+, RP-, SBR 2 presented with BM (2.6%) at initial diagnosis, with simultaneous lung, liver, bone and nodes metastasis. Four pts (4.8%) presented BM during follow up, among those two symptomatic pts (2.4%) as the only site of recurrence at 21 and 28 months. Median DFMS was 8.5 years and median OS was 16.7 years.

This is the first report of systemic imaging at initial diagnosis of HER2 positive locally BC pts. The results did not support initial brain imaging in this subset of pts. Only pts with symptoms seems to benefit from brain imaging. Further prospective study should be launched to confirm this observation.
Title: An automated DNA methylation assay (QM-MSP) for rapid breast cancer diagnosis in underdeveloped countries

Mary Jo Fackler¹, Bradley M Downs¹, Claudia Mercado-Rodriguez¹, Ashley Cimino-Mathews¹, Chuang Chen², Jing_Ping Yuan², Leslie M Cope¹, Andrew Kohlway³, Kriszten Kocmond³, Edwin Lai³, Jodi Weidler³, Kala Visvanathan¹, Christopher B Umbricht¹, Susan Harvey¹, Antonio C Wolff¹, Michael Bates³ and Saraswati Sukumar¹, ¹Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD; ²Wuhan University, Wuhan, Hubei, China and ³Cephied, Sunnyvale, CA.

Body: BACKGROUND: Underdeveloped countries reported 882,900 new cases of breast cancer and 324,000 deaths in 2012, likely to be a gross underestimation according to recent reports. Often, mammography screening is not available, primary care services are limited, and pathology and treatment services are available only in the regional hospitals. Because of the lack of access to diagnostic and treatment services, it is estimated that more than 90% of patients with breast cancer never present for medical treatment. To address this situation, an accurate, easy-to-perform diagnostic test appropriate for use in remote clinics is desperately needed. Johns Hopkins (JH) and Cepheid partnered to translate a robust Quantitative Multiplex Methylation-Specific PCR (QM-MSP) assay to an automated, cartridge-based system that provides quantitative measures of DNA methylation within hours of fine needle aspiration or core biopsy of image-detected suspicious lesions.

METHODS: With a goal of discriminating malignant from benign breast disease with high sensitivity and specificity, we evaluated 24 breast cancer-specific DNA methylation markers (selected through comprehensive methylome analysis) in 119 invasive ductal carcinomas and 186 benign breast tissues. QM-MSP was performed on sections of formalin-fixed paraffin-embedded (FFPE) tissues to quantify DNA methylation. The dynamic range and performance of quantitative methylation detection was tested using a subset of 9 genes in the cartridge-based system.

RESULTS: QM-MSP was performed in a Training set consisting of 93 tissues [n=43 IDC, n=50 benign lesions (25 usual ductal hyperplasia, UDH, and 25 papilloma)] from the US. We selected 9 DNA markers significantly (p<0.05) more methylated in malignant compared to benign lesions, which had low or no methylation. An independent Test set consisted of benign (n=26) and malignant (n=10) tissues (mostly Caucasian; JH Test Set). As a panel, the 9 markers were significantly more methylated in malignant than benign tissue (p<0.001), revealing a sensitivity of 90% and specificity of 92%, using a laboratory cutoff of 9.5 CMI units (900 unit scale) based on receiver operator characteristic statistics (ROC; p<0.0001, AUC=0.977). To determine if the markers characterized in the JH Test Set could perform as well in samples from a different geography, the panel was tested on 176 tissues from Wuhan, China (China Test Set). In this cohort (66 IDC and 110 benign tissues - 49 fibroadenoma, 19 benign cyst, 12 UDH, 30 papilloma), sensitivity was 89% and specificity was 89% for detection of breast cancer with ROC AUC=0.945. An advanced version of the cartridge with up to 12 methylated DNA markers is under development, thus far showing robust signals in cancer and low background in benign tissues. Current work at JH is focused on optimizing the technical performance of the cartridge.

CONCLUSIONS: We identified a panel of methylated DNA markers that discriminate malignant from benign breast lesions and built a prototype automated cartridge-based assay with promising sensitivity and specificity for breast cancer. Such an assay has the potential to aid in specimen triage in the pathology lab and provide fast, low cost and accurate diagnosis of breast cancer in LMIC settings.
Title: Detection ability of dedicated breast positron emission tomography for small-sized breast cancer

Satoshi Sueoka¹, Norio Masumoto¹, Mai Nishina¹, Yuri Kimura¹, Eri Suzuki¹, Noriko Goda¹, Shinsuke Sasada¹, Keiko Kajitani¹, Akiko Emi¹, Rumi Haruta¹, Takayuki Kadoya¹, Tsuyoshi Kataoka¹ and Morihito Okada¹. ¹Hiroshima University Hospital, Hiroshima, Japan.

Background: Whole body (WB) $^{18}$F-fluorodeoxyglucose positron emission tomography (PET) has a relatively poor spatial resolution (>1 cm), which limits the capability to detect small lesions. Therefore, small-sized breast cancers (≤1 cm) may not be visible on WB-PET. To overcome these limitations, dedicated breast PET (DbPET) has been developed to improve spatial resolution. DbPET enables detailed high-resolution images within the breast. We aimed to determine whether DbPET can detect small-sized breast cancer compared to WBPET.

Methods: We investigated 203 consecutive patients (217 tumors) (T1–3, N0–3a, M0) with breast cancer who underwent concurrent DbPET and WBPET between January 2016 and March 2017. All DbPET and WBPET images were semi-quantified based on standard uptake values. The diagnostic performance of each scanner was assessed in DbPET and WBPET. Tumors were classified based on pathological classification as follows: Tis, ductal carcinoma in situ (DCIS); T1a, ≤0.5 cm; T1b, 0.5–1 cm; and T1c, 1–2 cm; T2, 2–5 cm; T3, >5 cm. The sensitivities of DbPET and WBPET were compared in each size group.

Results: Table 1 shows the detection rate of breast cancer in WBPET and DbPET.

<table>
<thead>
<tr>
<th>Tumor size</th>
<th>DbPET Detection (-) n(%)</th>
<th>DbPET Detection (+) n(%)</th>
<th>WBPET Detection (-) n(%)</th>
<th>WBPET Detection (+) n(%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tis</td>
<td>6(14.6)</td>
<td>35(85.4)</td>
<td>18(43.9)</td>
<td>23(56.1)</td>
<td>0.0030</td>
</tr>
<tr>
<td>T1a</td>
<td>2(8)</td>
<td>23(92)</td>
<td>7(28)</td>
<td>18(72)</td>
<td>0.0594</td>
</tr>
<tr>
<td>T1b</td>
<td>2(6.5)</td>
<td>29(93.5)</td>
<td>10(32.3)</td>
<td>21(67.7)</td>
<td>0.0077</td>
</tr>
<tr>
<td>T1c</td>
<td>5(8.2)</td>
<td>56(91.8)</td>
<td>11(18)</td>
<td>50(82)</td>
<td>0.1038</td>
</tr>
<tr>
<td>T2</td>
<td>0(0)</td>
<td>57(100)</td>
<td>1(1.8)</td>
<td>56(98.2)</td>
<td>0.2375</td>
</tr>
<tr>
<td>T3</td>
<td>0(0)</td>
<td>2(100)</td>
<td>0(0)</td>
<td>2(100)</td>
<td>-</td>
</tr>
<tr>
<td>total</td>
<td>15(6.9)</td>
<td>202(93.1)</td>
<td>47(21.7)</td>
<td>170(78.3)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

. The overall detection rate in DbPET [93.1% (202/217)] was significantly higher than that of WBPET [78.3% (170/217)] (P < 0.001). For smaller tumors, DbPET was more sensitive than WBPET: Tis (85.4% vs. 56.1%), T1a (92% vs. 72%), T1b (93.5% vs. 67.7%), T1c (91.8% vs. 82%), T2 (100% vs. 98.2%), and T3 (100% vs. 100%). The sensitivity of DbPET was significantly higher than that of WBPET in Tis (P = 0.003) and T1b (P = 0.008) and tended to be higher than that of WBPET in T1a (P = 0.059).

Conversely, no significant differences were observed in T1c (P = 0.103) and T2 (P = 0.238).

Conclusion: The imaging sensitivity of DbPET was higher than that of WBPET. DbPET showed significant sensitivity in DCIS and tumors ≤1 cm, which is a weak point for WBPET. DbPET may serve as a new diagnostic modality to detect small-sized breast cancer.
Title: Internal mammary lymph node biopsy during free flap breast reconstruction: Accurate oncologic staging leads to change in adjuvant therapies

Oscar Ochoa¹, Steven Pisano¹, Minas Chrysopoulo¹, Peter Ledoux¹, Gary Arishita¹ and Chet Nastala¹. ¹PRMA Plastic Surgery, San Antonio, TX.

Body: Introduction: Accurate breast cancer staging, including nodal status, is essential for optimal management of adjuvant therapies leading to improved disease-specific and overall survival. Lymphatic drainage of the breast is known to involve both axillary and internal mammary (IM) lymph node basins. While IM nodal metastases represent advanced cancer stage, sampling is not routinely advocated due to relative lack of accessibility and the assumption that IM node positivity rarely alters adjuvant therapies. The current study analyzes the incidence of IM nodal metastases sampled during routine IM vessel exposure for free flap breast reconstruction and changes in adjuvant treatment.

Methods: A retrospective analysis of patients with positive IM lymph node biopsies at the time of free flap breast reconstruction following mastectomy between September 2008 and December 2015 was performed. Patients undergoing reconstruction following prophylactic mastectomy and reconstruction where IM vessels were not exposed were excluded. Prevalence of neoadjuvant therapies and previous lumpectomy with axillary lymph node (LN) sampling were recorded. Tumor size, location, and axillary LN status were obtained from final pathologic analysis. Prognostic factors and proliferative index (Ki-67) for the primary breast lesion were utilized to determine oncologic subtype. Change in adjuvant therapies (chemotherapy or external beam radiation) based solely on IM LN positivity was calculated.

Results: During the study period, 2057 patients fit study criteria. Twenty eight (1.3%) patients were found to have IM LN metastases and comprised the study population. Mean age of patients with positive IM metastases was 49 years with pre-reconstruction chemotherapy or radiation administered in 50% or 54% of cases, respectively. Five (18%) patients had previously undergone lumpectomy and axillary sampling prior to mastectomy and reconstruction for cancer recurrence. Mean tumor size was 3.1 cm (range 0.4 to 10 cm) with tumor location evenly distributed among all four quadrants. Although statistical significance between subtypes was not identified, luminal B (42.9 percent) was the most common subtype present. Ten (36%) patients had isolated IM lymph node metastases with negative axillary nodal disease. Patients with both axillary and internal mammary disease had larger lesions (4.4 cm vs. 2.5 cm), increased prevalence of pre-reconstruction chemotherapy (65 percent vs. 20 percent) and radiation (71 percent vs. 20 percent). Multivariate analysis did not identify any significant independent factors associated with isolated IM lymph node metastases. Ultimately, 17 (63%) patients had a change in their adjuvant therapy (additional chemotherapy or IM radiation therapy) based on positive IM LN disease.

Conclusion: Despite the low overall incidence of IM LN metastases, IM LN biopsy at the time of free flap breast reconstruction is recommended. In 36% of cases, nodal metastases were isolated to the IM nodes. Interestingly, a significant percentage (18%) of patients had previously undergone lumpectomy and axillary sampling. Identification of IM metastases significantly influenced adjuvant therapies in a majority of cases.
Using high-frequency ultrasound (20-80 MHz) to differentiate malignant vs benign breast tissue in surgical margins

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Body: The use of high-frequency (HF) ultrasound (20-80 MHz) to determine tissue pathology is an inexpensive and real-time tool for differentiation of surgical margin specimens from breast conservation surgery (BCS). The development of this method as an intraoperative tool for BCS would greatly reduce the rate of re-excisions due to positive breast tissue margins. HF ultrasound was previously used in a 17-patient study to determine differences in breast tissue pathology of 34 surgical margins. Results from this pilot study demonstrated high accuracy, specificity, and sensitivity in the HF ultrasound data, and showed the immense potential of this method as a breast cancer detection tool. A large-scale validation study of this method was subsequently conducted using 349 margin specimens taken from 73 patients during BCS. Specimens ranged from 1-5 cm in length and width, 0.2-1.6 cm in thickness, and did not require any additional procedures or resection that affected the patient or surgical outcome. Each specimen was tested with HF ultrasound immediately following resection and then forwarded to pathology. Through-transmission data were collected from the specimens using two broadband, single-element transducers with a 50-MHz peak frequency (Olympus NDT, V358-SU), a HF square-wave pulser/receiver (UTEX, UT340), and a 1-GHz digital oscilloscope (Agilent, DSOX3104A). Peak density (the number of peaks and valleys in the 20-80 MHz frequency spectra range) and attenuation data were calculated from the ultrasonic spectra and waveforms, respectively. Peak density and attenuation correlate directly to tissue malignancy, and thresholds for these two parameters for differentiating benign vs malignant tissue were determined using Fisher’s Exact Test applied to the previously collected pilot study data. The peak density and attenuation results were then combined using a multivariate analysis. Since the ultrasonic measurements were collected on a per position basis (1-5 positions per margin), but the histopathology results were reported on a per specimen basis, the statistical measures for the ultrasonic results were calculated using two methods. First, the statistical measures were calculated based on a per position basis, where the pathology of each position was determined by the pathology results for the entire specimen. Second, the statistical measures were calculated based on a per specimen basis, where only one measurement position on each margin was selected (based on the highest peak density value) to correlate to the specimen pathology. The results of the first approach (per position basis) showed a sensitivity of 82.6%, a specificity of 72.3%, and an accuracy of 72.7%. The results of the second approach (per specimen basis) showed a sensitivity of 82.6%, a specificity of 61.7%, and an accuracy of 63.0%. The results of this study show potential for a rapid and inexpensive method of determining pathology of breast tissue surgical margins during BCS. This work was supported by funds from the Elsa U. Pardee Foundation, the Eppley Foundation for Research, the Western Alliance for Expanding Student Opportunities, the Governor’s Office of Economic Development of the State of Utah, the University of Utah, and Utah Valley University.
Title: Differences in serum miRNA signatures in women with pathology-confirmed breast cancer, benign breast diseases and healthy individuals with no cancer history

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Body: [INTRODUCTION] Mammography is the principal non-invasive imaging method of screening for breast cancer around the world. Mammography has been shown to be effective in reducing breast cancer deaths in randomized studies (Tabar et al., 2011 and Duffy et al., 2002). However, screening mammography can result in false positives that require subsequent interventions such as biopsy or interval repeat mammography.

Recently, circulating micro RNAs (miRNAs) have been cited as promising minimally invasive markers for breast cancer as well as for various other types of malignancies (Shimomura et al., 2016; Lawrie et al., 2008; Wang et al., 2009; Zhang et al., 2010; Mitchell et al., 2008). In this study, we have developed a new molecular diagnostic platform for the measurement of circulating (serum) miRNAs to assist with the management of women with abnormal screening mammograms.

[METHODS] Peripheral venous blood samples were collected in BD Red/Gray gel barrier tubes from patients with untreated breast cancer, patients with benign breast diseases and healthy individuals with no cancer history. The processing from blood to serum was done within six hours of collection and stored at -80°C. Total RNA was extracted from 300μL of the resulting serum using 3D-Gene®RNA extraction reagent (Toray Industries, Inc). Comprehensive miRNA expression analysis was then performed using 3D-Gene® Human miRNA Oligo Chip (Toray Industries, Inc). The average of three pre-selected internal control miRNA (miR-149-3p, miR-2861 and miR-4463) was used to normalize the signals across the different microarrays tested.

[RESULTS] All serum specimens used in this study were from subjects enrolled at US clinical sites (83% Caucasian, 15% African American, 2% Asian and others). One hundred fifteen (115) specimens (breast cancer: 40, healthy: 45, benign breast lesions: 30) were selected as a training cohort and used to develop comprehensive discriminants with 1–5 miRNAs using Fisher's linear discriminant analysis. The analysis identified a combination of five miRNAs that could detect breast cancer, and the resulting discriminant equation showed a sensitivity of 90.5%, specificity of 89.5% and accuracy of 89.8%. As test cohorts, (A) 381 subjects (breast cancer: 63, healthy: 160, benign breast lesions: 152), independent from subjects in the training cohort, and (B) 35 subjects who had been referred for breast biopsy were tested. The discrimination performance of the equation for each breast cancer stage (Stage 0: 1, Stage 1: 20, Stage 2: 17, Stage 3: 14, Stage 4: 11) was examined in the test cohorts. Also, information from breast cancer types as well as from benign breast lesions have been used to assess the performance of the discriminant score. The results of these analyses along with a discussion of the potential significance and plans for expanded clinical trials will be presented.
Title: Rapalog everolimus induces G1 cell cycle arrest through autophagy-mediated protein degradation of cyclin D1 in breast cancer cells

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Body: Rapamycin analogs (rapalogs) inhibit mammalian target of rapamycin (mTOR) and are known to cause induction of autophagy and G1 cell cycle arrest. However, it remains unknown whether rapalog-induced autophagy plays a critical role in its regulation of cell cycle. We, for the first time, found that rapalog everolimus could stimulate autophagy-mediated Cyclin D1 degradation in breast cancer cells. Inhibiting mTOR with everolimus rapidly increased the degradation of Cyclin D1 in MCF-10DCIS.COM and MCF-7 cells. 3-Methyladenine (3-MA), a classic autophagy inhibitor, could attenuate everolimus-induced Cyclin D1 degradation. Furthermore, knockdown of autophagy related gene 7 (Atg-7) could also repress everolimus-triggered degradation of Cyclin D1 in MCF-10DCIS.COM. Moreover, everolimus-induced autophagy occurred earlier than its induction of G1 arrest and cell death. Blockade of autophagy attenuated everolimus-induced G1 cell cycle arrest. These preliminary data support the conclusion that the autophagy induced by rapalogs in breast cancer cells appears to cause Cyclin D1 protein degradation resulting in G1 cell cycle arrest. Our findings contribute to our knowledge of the interplay between autophagy and cell cycle regulation mediated by mTOR signaling and Cyclin D1 regulation.
CDK6 might be a key factor for efficacy of CDK4/6 inhibitor and the hormone sensitivity following acquired resistance

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Body: Background: CDK4/6 inhibitors have received FDA breakthrough therapy designation as 1st line treatment for advanced estrogen receptor positive breast cancer patients. However, the benefit offered by CDK4/6 inhibitors is individually different and furthermore acquired resistance to the drugs is monumental challenges. It is urgent need to search for the biomarker and understand the drug sensitivity and its alteration after acquired resistance.

Results: To identify the efficacy of CDK4/6 inhibitors, we assessed IC50 of ribociclib in several cell lines. Luminal cell lines (MCF-7, T-47D) exhibited lower ribociclib IC50 than HER-2 (SK-BR-3) and triple negative cell lines (MDA-MB-231, BT-20). Immunoblot analysis of Luminal cell lines showed extremely lower levels of CDK6 compared with others. CDK6-transfected MCF-7 by means of expression vector reduced the sensitivity equivalent to MDA-MB-231 not only to ribociclib but also to palbociclib and abemaciclib. Protein level of ERα in CDK6-transfected MCF-7 stayed unchanged and fulvestrant sensitivity was unaltered as well.

Subsequently, we detect the efficacy of ribociclib in hormone resistant cell lines. Estrogen deprivation-resistant (EDR) cells (EDR1:ER-positive, EDR2:ER-negative) and fulvestrant resistance (MFR) cells (loss of ER expression) established from MCF-7 maintained ribocilib sensitivity to the same degree with MCF-7. No marked difference in IC50 was observed between EDR1/2 and MFR, and CDK6 expressions were comparable to MCF-7. These results suggest that high level of CDK6 expression weaken the sensitivity to CDK4/6 inhibitors. The inhibitors would provide more effective benefits to tumors expressing lower level of CDK6 than the higher, independent of hormone sensitivity.

To understand the characteristics in acquired resistance, ribociclib resistant cell lines (RIBR1/2) were established from EDR1 by long-term culture with ribociclib. RIBR designed lower level of p21, p27 and ERα by immunoblot analysis. EDR1 were promoted cell growth by estrogen addition, while RIBR were not. Further, ER activity of RIBR was intensely decreased, and mRNA levels of the ER target genes, PgR and EGR3 were also decreased. Therefore, the responsiveness to tamoxifen and fulvestrant were lost. On the other hand, PI3K inhibitor and mTOR inhibitor suppressed cell growth to the same extent as EDR1, suggesting that RIBR were reduced ER dependence and remain reliant on PI3K/Akt/mTOR pathway.

Conclusion: The possibility of CDK6 as a biomarker is corroborated by the finding that low level of CDK6 expression is positively correlated with the efficacy of CDK4/6 inhibitor. Further ER dependence had decreased after acquired CDK4/6 inhibitor resistance whereas the dependence on PI3K/Akt/mTOR pathway still remain, indicating the inhibition of PI3K/Akt/mTOR pathway would be amenable to therapeutic target.
Title: Interactions between adipocytes and breast cancer cells stimulate cytokine production and drive Src/SOX2/miR-302b mediated malignant progression

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Body: Consequences of the obesity epidemic on cancer morbidity and mortality are not fully appreciated. Obesity is a risk factor for many cancers, but the mechanisms by which it contributes to cancer development and patient outcome have yet to be fully elucidated. Here, we examined the effects of coculturing human-derived adipocytes with established and primary breast cancer cells on tumorigenic potential. We found that the interaction between adipocytes and cancer cells increased the secretion of proinflammatory cytokines. Prolonged culture of cancer cells with adipocytes or cytokines increased the proportion of mammosphere-forming cells and of cells expressing stem-like markers in vitro. Furthermore, contact with immature adipocytes increased the abundance of cancer cells with tumor-forming and metastatic potential in vivo. Mechanistic investigations demonstrated that cancer cells cultured with immature adipocytes or cytokines increased the proportion of mammosphere-forming cells and of cells expressing stem-like markers in vitro. Furthermore, contact with immature adipocytes increased the abundance of cancer cells with tumor-forming and metastatic potential in vivo. Mechanistic investigations demonstrated that cancer cells cultured with immature adipocytes or cytokines activated Src, thus promoting Sox2, c-Myc, and Nanog upregulation. Moreover, Sox2-dependent induction of miR-302b further stimulated cMYC and SOX2 expression and potentiated the cytokine-induced cancer stem cell-like properties. Finally, we found that Src inhibitors decreased cytokine production after coculture, indicating that Src is not only activated by adipocyte or cytokine exposures, but is also required to sustain cytokine induction. These data support a model in which cancer cell invasion into local fat would establish feed-forward loops to activate Src, maintain proinflammatory cytokine production, and increase tumor-initiating cell abundance and metastatic progression. Collectively, our findings reveal new insights underlying increased breast cancer mortality in obese individuals and provide a novel preclinical rationale to test the efficacy of Src inhibitors for breast cancer treatment.
Title: Targeting vesicular trafficking machinery for breast cancer therapeutics

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Body: The Rab GTPase family is a key regulator of vesicular import, transport and export. Deregulation of vesicular trafficking promotes undesirable intercellular cross talk via exosomes that contribute to cancer pathophysiology. Preclinical and clinical studies underscore a role for exosomes and their cargo in breast cancer development, progression, metastasis, and resistance to therapy. While many studies have approached the role of exosomes in cancer pathophysiology, the 'rules' governing their production and cargo sorting represents a major unexplored gap in knowledge. Exosomes arise from internal budding of specific endosomal compartments. Rab11 GTPase family members comprised of Rab11a, Rab11b and Rab25 are critical regulators of endocytic cargo trafficking specifically of apical and recycling endosomes. Previously, our lab has shown that Rab25 is amplified and contributes to oncogenesis in both ovarian and Luminal B breast cancers. However, Rab25 is lost and when re-expressed ectopically, it inhibits invasion and migration in Claudin Low tumors suggesting a cell-lineage and context dependent function. Importantly, while in Luminal B cancer, Rab25 and its downstream effector RCP (Rab coupling protein), collaborate in driving oncogenesis, in Her2+ cancers, RCP expression is protective. Further our group recently uncovered the presence of a stroma enriched “reactive protein signature” in ER+/Her- group that is indicative of good outcome for patients. Indeed, many of the “reactive signature” proteins are reported in exosomal cargo by others. Taking together, our ongoing study utilizes a panel of breast cancer cell lines representing ER+ve/Her2+; ER+/Her-, ER-/Her2+, and ER-/Her- to evaluate the role of Rab11 family in modulating vesicular trafficking that alters the exosome biology and how that may contribute to tumor-stroma communications. Our preliminary results show that indeed over expression of Rab25 differentially alters size and number of extracellular vesicles in Luminal B cancers when compared to the Claudin Low subtype. Using a novel class of peptides that interrupt the cellular functions 25, we are investigating if Rab25 expression can be associated with an exosomal cargo profile in each subtype of breast cancer, which could then serve as a biomarker for tumor-stroma interaction.
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Title: Attenuation of RNA polymerase II pausing mitigates BRCA1-associated R-loop accumulation and tumorigenesis

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Body: Most BRCA1-associated breast tumours are basal-like yet originate from luminal progenitors. BRCA1 is best known for its functions in double-strand break repair and resolution of DNA replication stress. However, it is unclear whether loss of these ubiquitously important functions fully explains the cell lineage-specific tumorigenesis. In vitro studies implicate BRCA1 in elimination of R-loops, DNA-RNA hybrid structures involved in transcription and genetic instability. Here we show that R-loops accumulate preferentially in breast luminal epithelial cells, not in basal epithelial or stromal cells, of BRCA1 mutation carriers. Furthermore, R-loops are enriched at the 5’ end of those genes with promoter-proximal RNA polymerase II (Pol II) pausing. Genetic ablation of Cobra1, which encodes a Pol II-pausing and BRCA1-binding protein, ameliorates R-loop accumulation and reduces tumorigenesis in Brca1-knockout mouse mammary epithelium. Our studies show that Pol II pausing is an important contributor to BRCA1-associated R-loop accumulation and breast cancer development.
Title: mRNA expression of PTK6V1 and PTK6V2 in human breast cancer

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Body: BACKGROUND:
Protein tyrosine kinase 6 (PTK6) is an intracellular protein that is upregulated in several human cancers and its localization to the plasma-membrane facilitates its various oncogenic roles in breast cancer cell proliferation, survival, and migration. The full length protein (PTK6V1) and alternative splice variant (PTK6V2) seem to play different roles with the latter being negative regulator of the former.

In this study, the level of mRNA expression of PTK6V1 and PTK6V2 were assessed in normal and malignant breast tissue using real time Q-PCR in a cohort of women with breast cancer and correlated to conventional clinico-pathological parameters and clinical outcome.

MATERIALS AND METHODS:
Breast cancer tissues (n = 127) and normal background tissues (n = 33) were collected immediately after excision during surgery. Following RNA extraction, reverse transcription was carried out and transcript levels were determined using real-time quantitative PCR and normalized against beta-actin expression. Transcript levels within the breast cancer specimens were compared to the normal background tissues and analysed against TNM stage, nodal involvement, tumour grade and clinical outcome over a 10 year follow-up period.

RESULTS:
The median copy number of transcripts of PTK6V1 were higher in malignant compared with normal breast tissue (23 vs.7) and overall increased with advancing tumour stage (374 vs. 13 for TNM3 vs. TNM1 respectively p=0.019 and 374 vs.23 for TNM3 vs. TNM2 p=0.0244). however these associations did reach statistical significance. PTK6V1 levels were associated with oestrogen receptor (ER) positivity (p = 0.061). The transcript levels were significantly higher in patients who developed recurrence (p=0.03) or died of breast cancer (p=0.003). PTK6V2 transcript levels were generally higher in normal breast tissue than in malignant tissues and decreased with increasing tumour stage and grade however these associations did not reach statistical significance. After a median follow up of 10 years, there was a trend for higher PTK6V2 expression to be associated with longer overall survival (OS) and disease free survival (DFS). PTK6V2/PTK6V1 ratio was a significant predictor of OS.

Conclusions
Our observations suggest that the two variants of PTK6 play opposing roles in mammary oncogenesis. These findings could have prognostic and therapeutic implications.
Title: Discistronic reporter screen for internal ribosome entry site (IRES) - mediated translational regulation of truncated p110 ERBB2 isoform

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Background:
We and others demonstrated that truncated p110 ERBB2 (p110 t-ERBB2, also as 611CTF) is a hyperactive truncated ERBB2 isoform capable of increasing cell migration and invasion in multiple cell types in vitro, and induction of human breast epithelial cell (HMLE) xenograft formation in vivo [Ward, et al., Oncogene (2013) 32, 2463–2474]. p110 t-ERBB2 arises through alternative initiation of translation from methionine 611, however, it is unclear how regulation of its expression may be achieved. mRNA structural elements termed internal ribosome entry sites (IRESs) can initiate translation in a cap-independent manner when canonical cap-dependent translation is severely compromised. By cloning the EGFR 5' untranslated region (UTR) between the Renilla and firefly luciferase open reading frames of pRF, Webb, et al. have reported human EGFR 5' UTR sequence can initiate expression of a downstream open reading frame via an IRES [Oncogenesis (2014) 3, e134]. Therefore, we sought to identify presence of a putative IRES within the 5'UTR ERBB2 mRNA which might mediate alternative ERBB2 protein translation initiation under stress conditions and promote p110 t-ERBB2 biosynthesis.

Methods:
Discistronic reporter pRF was used as the backbone vector to detect IRES activity. Promoter-less vector pRFΔP were constructed by removing SV40 promoter via restriction digestion. The HER2 mRNA 5'UTR (from both variant 1 and variant 3) and several overlapping sequences from full length ERBB2 (p185 ERBB2) start codon to p110 t-ERBB2 start codon were cloned between the Renilla and firefly luciferase open reading frames of pRF and pRFΔP, then the resultant construct were transiently transfected into different cell lines (BT474, SK-BR3, MCF-7, HeLa, CHO). Three control constructs pRF (empty vector control), pRF-Tub (negative control, containing βtubulin 5'UTR, which lacks IRES activity) and pRF-myc (positive control, containing the well-characterized c-myc IRES) were parallel-transfected. Luciferase expression was then quantified using a Dual Luciferase Assay Kit (Promega, Madison, WI, USA) following manufacturer's instructions. Parallel western blot analysis and qRT-PCR were also conducted.

Results:
In this report, we demonstrate that in BT474 and SK-BR3 cells, no IRES activity was detected within human ERBB2 5'UTR sequences under non-stressed conditions, or under serum-starvation, hypoxic conditions or thapsigargin-induced endoplasmic reticulum stress -- conditions when global translation was compromised. The construct pRF+265/+1561 (within ERBB2 mRNA coding sequence, 5' to the p110 t-ERBB2 start codon) displayed a 10-21 fold increase in firefly/Renilla activity when compared with the empty control pRF and negative control pRF-Tub, consistent with the possibility that the region between +265/+1561 may contain a cryptic promoter.

Conclusions:
These data are inconsistent with the hypothesis that a 5'UTR IRES-mediated mechanism is involved in the translation of p110 t-ERBB2 isoform, and that other mechanisms are operative in alternative translational regulation/biosynthesis of p110 ERBB2 isoform.
Title: Comprehensive analysis of the DNA damage repair and maintenance pathways that regulate TNBC sensitivity to replication stress

Abena B Redwood¹, Sahil Seth¹, Shirong Cai¹ and Helen Piwnica-Worms¹. ¹MD Anderson Cancer Center.

Body: Agents that induce replication stress, such as inhibitors of Chk1 or ATR, are advancing in clinical development and are being tested for treatment of various solid tumors, including triple-negative breast cancer (TNBC). While the preclinical data are encouraging, additional studies are needed to predict with precision (i) which patients will most likely benefit from these inhibitors, (ii) the genetic and proteomic contexts in which these inhibitors will provide maximum therapeutic benefit as a single agent, or require additional sensitization via combination with a targeted- or chemotherapeutic agent, and (iii) exactly which targeted/chemotherapeutic agent will provide maximum therapeutic benefit for combination with replication stress inducers. To address these challenges in TNBC, we have attempted to gain a comprehensive understanding of how the DNA damage response pathways regulate TNBC cell survival in response to Chk1 inhibitors, by performing high throughput loss-of-function screens.

We have identified genes whose loss induces death of TNBC cells in the presence of (1) CHK1i alone, (2) chemotherapy alone or (3) CHK1i plus chemotherapy. In addition, given the role of TP53 as the most frequently mutated gene in TNBC, we also determined whether distinct vulnerabilities could be identified in TNBC cells that are p53-proficient versus p53-deficient. Thus, we have also identified the top synthetic lethal interactions that are either common to both p53-proficient and p53-deficient TNBC, or unique to p53-deficient TNBC; we are currently performing in vitro studies to validate the identified mechanisms. We anticipate these studies to be applicable to other agents that induce replication stress and cell cycle checkpoint bypass. Ongoing in vivo preclinical studies, which utilize patient-derived xenografts (PDXs) of TNBC to validate these findings are expected to impact patient selection for clinical trials, and also allow us to predict which chemotherapeutic agents will be most effective for combination with different cell-cycle checkpoint inhibitors.
Title: Functional homologous recombination REpair CAPacity (RECAP) test in metastatic breast cancer biopsies

Tumors of germline BRCA1/2 mutated carriers cannot repair DNA double strand breaks (DSB) due to homologous recombination deficiency (HRD), rendering them highly sensitive to Poly ADP Ribose Polymerase (PARP) inhibitors and DSB inducing chemotherapy. Although evidence is emerging that the use of these therapies could be extended beyond germline BRCA1/2 mutated carriers, a robust validated test to detect HRD tumors is lacking. We have developed a functional HR assay exploiting the formation of RAD51 foci in proliferating cells after ex vivo irradiation of fresh primary breast cancer (BrC) tissue: the RECAP test. Here, we investigated feasibility of this test on small biopsies from metastatic BrC lesions.

Methods Patients with advanced or recurrent BrC with easily accessible metastases were eligible. Patients with pulmonary or bone metastases were excluded due to risk of pneumothorax and technical issues with calcifications. Biopsies were collected in customized DMEM/F12 medium, irradiated with 5Gy and cultured for 2 hours. Final test outcomes were available within 1 week after the biopsy procedure. The primary endpoint was the proportion of patients with a successful test result defined as either less than 20% (i.e. HRD), 20-50% (i.e. HR intermediate), or more than 50% (i.e. HR proficient) of S/G2 phase cells showing RAD51 foci. Using a Simon 2-stage design (p0=60%, p1=80%, α=0.1, β=0.1), 38 patients were required. When at least 27 had a successful test result, we decided to further develop this test.

Results 51 patients enrolled in the RECAP trial. Core needle biopsies were collected from liver (n=16), breast (n=4), lymph nodes (n=13) and other sites (n=6), as well as punch biopsies from skin metastases (n=5). In 6 cases a biopsy could not be obtained; 1 patient was excluded as the biopsy appeared to be a metastasis of a neuroendocrine tumor. Of 44 obtained biopsies, 42 biopsies contained tumor cells. Successful test results were obtained in 40 of the 42 metastatic biopsies (95%, CI 89-100%). The reason for the two unsuccessful tests, both derived from ER+/PR+/HER2-liver metastases, was absence of tumor cells in S-phase. Success of the test in biopsies was independent of BrC subtype (p=0.12), histological grade (p=0.65), receptor status (p=0.74), biopsy type (p=0.82) and metastatic site (p=0.78). We found that 25 metastatic lesions were HR proficient, 13 were HR deficient and 2 were HR intermediate. Germline BRCA testing showed mutations in 6/13 HRD lesions. Somatic and epigenetic analyses are ongoing.

Conclusion In this study, we enhance the diagnostic potential and clinical applicability of the functional RECAP test by demonstrating novel feasibility on metastatic biopsies from BrC patients. Robustness of the test is shown, as its applicability extends from core needle biopsies from different metastatic sites to punch biopsies from skin metastases. Comparison of HRD status of primary versus metastatic tumors and among BrC subtypes will be presented at the conference.
**2017 San Antonio Breast Cancer Symposium**

**Publication Number:** P6-06-03

**Title:** Molecular mechanisms contributing to BRCA1 restoration in a subset of germline BRCA1 mutated tumors with homologous recombination repair functionality

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**Body:**

**Introduction:** Tumors from patients with pathogenic germline BRCA1 (gBRCA1) variants have impaired BRCA1 function and subsequent homologous recombination repair (HRR) deficiency that sensitizes to DNA damaging agents or PARP inhibitors. In chemotherapy-and/or PARP inhibitor-resistant gBRCA1 tumors, recovery of BRCA1 open reading frame by secondary mutation, or the expression of hypomorphic BRCA1 isoforms including the BRCA1 RINGless protein and the ∆11q BRCA1 isoform have been described. We have comprehensively profiled the BRCA1 status in a set of gBRCA1 tumors with HRR capacity, including genomic and mRNA characterization and subcellular localization of BRCA1 protein.

**Material:**

1) Cancer models: we selected 11 gBRCA1 displaying RAD51 nuclear foci, as a surrogate marker of HRR functionality from a panel of gBRCA1 patient-derived xenografts (PDXs). Nine out of the eleven PDXs derived from triple negative breast cancer (TNBC); the remaining two were high-grade serous ovarian cancer models. 2) Clinical samples: 16 archival FFPE samples from gBRCA1 TNBC patients were employed, half of which matched the selected PDXs.

**Methods:**

1) Mutations in BRCA1 and loss of heterozygosity (LOH) were evaluated in all gBRCA1 models by BRCA1 amplicon sequencing. 2) Intragenic and allelic copy number changes of BRCA1 were assessed by MLPA. 3) BRCA1 mRNA expression was measured by RT-qPCR. 4) BRCA1 mRNA isoforms were analyzed by capillary electrophoresis of FAM-labeled amplicons. 5) BRCA1 nuclear foci formation was examined by immunofluorescence (IF) using antibodies against BRCA1, directed to the N-terminus, the region encoded by the BRCA1 exon 11, or the C-terminus.

**Results:**

No in-frame secondary mutations in BRCA1 were identified. One PDX derived from a carrier with a whole BRCA1 deletion had no BRCA1 alleles. The remaining gBRCA1 models presented LOH with loss of the wild type allele. In contrast, BRCA1 protein expression and nuclear foci formation was identified by IF in 8/11 (73%) of gBRCA1 PDXs with HRR functionality. Five gBRCA1 models (45%) displayed copy-neutral LOH (cnLOH) for BRCA1. Models with BRCA1 copy gains showed a trend towards higher gene expression. The results of the available archival samples mirrored those of their matched PDXs in all cases. Analysis of BRCA1 splicing variants demonstrated high expression of the ∆11q BRCA1 splicing isoform among the PDXs models carrying a gBRCA1 mutation in exon 11.

**Conclusions:** In gBRCA1 tumors with HRR capacity, BRCA1 functionality unveiled by BRCA1 nuclear foci formation was a frequent event. Results were confirmed in available archival samples of the corresponding patients. Various molecular mechanisms contributed to BRCA1 functionality beyond secondary mutations, including cnLOH and alternative splicing that may contribute to enhance gene expression and hypomorphic BRCA1 proteins. These factors could correlate with response to chemotherapy or PARP inhibitors and guide the selection of novel therapies for gBRCA1-related cancers.
Title: Targeting replication stress in triple negative breast cancer treatment regimen: An emerging approach

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Body: Triple-negative breast cancers (TNBCs) represent aggressive heterogeneous subtype of breast cancer with poor clinical outcome. TNBCs have been reported to have high levels of replication stress due to i) various oncogene activations (C-myc or EGFR) ii) germline BRCA mutations iii) “BRCAness” in the absence of BRCA mutations in sporadic TNBCs. Replication stress is known to cause genomic instability, promote tumorigenesis and plays a critical role in therapy resistance in TNBCs. Therefore, targeting replication stress has emerged as an effective approach for better TNBC treatment through further downregulation of the remaining checkpoints to induce catastrophic failure of TNBC cells proliferation. Herein, we evaluated the anticancer efficacy of Carbazole Blue (CB), a synthetic analogue of Carbazole, on TNBC cells growth and progression. Our results demonstrated that CB inhibits short and long term viability of TNBC (MDA-MB-231, MDA-MB-468 and BT549) cells in a dose dependent manner without affecting normal mammary epithelial (MCF-10A) cells. In addition, CB treatment significantly reduced proliferation of TNBC cells, as evidenced by the BrdU proliferation assay. Consistent with this, our results further demonstrated that CB treatment induced G1/S cell cycle arrest and apoptosis in TNBCs. Importantly, systemic delivery of CB using nanoparticle-based delivery approach suppressed breast cancer growth without inducing toxicity, in preclinical orthotopic xenograft and PDX mouse models of TNBC. Furthermore, our gene microarray analysis revealed that CB treatment modulates the expression and activity of several genes known to be involved in DNA replication (CDC6, CDT1, MCMs, Claspin, POLE and PCNA) and associated DNA repair machinery such as (XRCC3, Exo1 and RAD51), which play pivotal roles in replication stress. Our results for the first time highlight the potential use of CB as a novel and potent therapeutic agent for treating TNBCs. As exploiting replication stress to treat cancer is gaining major interest, compound/s that may induce replication stress and inhibit DNA repair ability of cancer cells, has immense translational potential.
Analysis of BRCA2 function in the *Xenopus laevis* model system

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Body: Basic research on proteins or processes known to be implicated in cancer is the cornerstone for the development of efficient diagnostic and therapeutic tools. Such research should ideally span across disciplines and employ multiple complementing experimental systems.

BRCA1 and BRCA2 are the major breast and ovarian cancer susceptibility genes. Since their discovery over two decades ago, the products of both genes have been extensively studied and implicated in various cellular processes, most notably in DNA damage response and repair via homologous recombination. This knowledge has provided the rationale for therapeutic targeting of DNA repair pathways in BRCA1- and BRCA2-mutated cancers. Our understanding of how BRCA1 and BRCA2 operate at the molecular level to prevent breast and ovarian cancerogenesis is, however, incomplete. Further advancement is needed to inform the development of more specific prevention and treatment modalities.

We have previously shown that BRCA1 and its partner protein, BARD1, are conserved in non-mammalian species and we pioneered the use of *Xenopus laevis* as a model to study the function of the BRCA1-BARD1 heterodimer (Joukov et al., PNAS 2001; Joukov et al., Cell 2006). While *Xenopus* embryos represent an excellent system for developmental studies, cell-free *Xenopus* egg extracts offer unique opportunities for detailed biochemical investigations. Such extracts can oscillate between interphase and mitosis, thus allowing faithful recapitulation of many complex cellular processes, including nucleus formation, DNA replication and repair, bipolar spindle assembly, and chromosome segregation. The function of a protein of interest can be assessed and elucidated in extracts using immunodepletion, specific inhibitors, or dominant negative mutants.

Here, we report the cloning of the full-length *Xenopus laevis* BRCA2 cDNA and the initial characterization of the endogenous BRCA2 protein using both *Xenopus* embryos and egg extracts. We found that BRCA2 is stockpiled in *Xenopus* oocytes and expressed throughout the embryonal development. In addition to the maternal BRCA2 protein, a shorter zygotic BRCA2 isoform is detected in *Xenopus* embryos after the midblastula transition. By immunodepletion and immunoprecipitation experiments we demonstrate that most of BRCA2 in the egg cytoplasm is bound to the Rad51 recombinase and that a fraction of BRCA2 interacts with BRCA1. We also found that both BRCA2 and Rad51 associate with chromatin in S phase and that the chromatin-bound BRCA2 is phosphorylated in an ATM/ATR-dependent manner. Consistent with observations made in other experimental systems, it appears that BRCA2, although not required for DNA replication in unperturbed egg extracts, is essential for Rad51 loading onto S phase chromatin.

Our studies indicate that the role of BRCA2 as a cofactor of Rad51 is conserved in *Xenopus laevis* and provide the groundwork for the use of *Xenopus* egg extracts as a biochemically tractable system to dissect the molecular function of the BRCA2 tumor suppressor and its partner proteins. Lessons learned in *Xenopus laevis* could then be translated into mammalian systems and ultimately be used to aid development of tailored diagnostic and therapeutic strategies.
Title: Immunogenomics approach elucidating clinical significance of DNA repair genes and tumor infiltrating immune cells in breast cancer

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Body: Backgrounds: Evading the immune system is one of the Hallmarks of Cancer. Indeed, tumor infiltrating immune cells has been shown to play critical roles in suppression of cancer progression. Genetic aberration of DNA repair genes is known to increase immunogenicity in breast cancer. However, the patient survival relevance of tumor infiltrating immune cells in regard to DNA repair genes has not yet elucidated in large cohort of breast cancer patients. We hypothesized that DNA repair gene deficiency is related to increased global genomic instability that leads to increased mutation burden, which recruits infiltrating immune cells to tumor microenvironment that result in better prognosis of breast cancer.

Patients and Methods: Integrated and unbiased transcriptomics approach was conducted on genomic and clinicopathological information of 3614 breast cancer patients. We utilized The cancer Genome Atlas (TCGA) and the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) to evaluate the association between the aberration of DNA repair genes and tumor infiltrating immune cell composition in breast cancer tumors, as well as its significant clinical relevance, utilizing bioinformatics and biostatistics pipelines.

Results: Low expression level of double-strand break repair genes; BRCA1, PRKDC, and RECQL4, demonstrated significantly better prognosis in TCGA cohort (p=0.018, p=0.036, and p=0.0002, respectively). This result was consistent in METABRIC cohort (p=0.021, p=0.00021, and p<0.000001, respectively). Utilizing CIBERSORT system that estimate the fraction of 22 immune cell types, we found that low expression of BRCA1 significantly associated with high levels of CD8 positive cell composition in both cohorts (TCGA, p=4.67E-08; METABRIC, p=0.0038), which implicate that tumor infiltrating lymphocytes are attracted to BRCA1 low expressing tumors. Further, low expression of BRCA1 showed significantly better survival in HER2 positive subtype population, but not in the other populations (TCGA, p=0.027; METABRIC, p=0.13). Finally, significantly poor prognosis was observed in breast cancers low in immune-response markers; PD-1, PD-L1, TIM3, LAG3, and CTLA4, in combination with high expression of BRCA1 (p=0.0016, p=0.0041, p=0.015, p=0.0041, and p=0.0043, respectively), which is in agreement with the dogma that intact DNA repair induce less immune-response that result in worse survival.

Conclusions: We conclude that our immunogenomics approach identify the interplay between DNA repair genes, especially gene expression of BRCA1, and tumor infiltrating immune cells, and it could have significant prognostic relevance in breast cancer.
Bisphenol A treatment induces hyperplasia in primary and stem cell-generated mammary glands from pregnant mice

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Body: Breast cancer is a commonly diagnosed cancer of pregnancy. However, how endogenous and exogenous endocrine factors may contribute to the development of pregnancy-associated mammary tumorigenesis is not clear. There is growing evidence that mammary stem cells (MaSCs) may initiate neoplastic transformation when dysregulated in mouse models. We investigated the effect of the environmental endocrine disruptor bisphenol A (BPA) on mouse mammary gland morphology, epithelial cell composition, pre-neoplastic lesions, and the regenerative function of MaSCs. Pregnant FVB mice with GFP transgene on Day E8.5 were implanted with osmotic pumps that constantly release BPA at 0, 25 or 250 ng/kg/day for 28 days and the mice were euthanized one month after weaning. In agreement with the literature, we observed an abnormality of the morphology of the mammary gland after BPA treatment characterized by higher duct density and abnormal secondary and tertiary branching. Quantification of percent hyperplastic mammary ducts in H&E-stained tissue slides revealed a significant increase of ducts with hyperplastic lesions after BPA treatment, particularly with the low dose. To investigate the effects of BPA treatment on MaSCs, we used enzyme digestion to isolate the CD24hi/CD49f+ luminal epithelial cells (also termed as colony forming cell or CFC) and the CD24+/CD49fhi basal epithelial cells (also termed as mammary repopulating unit or MRU) from mammary gland tissues by FACS and found no significant difference in percent of luminal or basal cell population after BPA treatment. Because the basal cells are enriched with MaSCs that can form mammospheres in suspension culture and subsequently form solid 3D organoids when cultured in Matrigel, we transplanted the solid 3D organoids into cleared mammary fat pads of syngeneic FVB mice and immune-compromised nude mice to examine how BPA treatment might alter MaSC function. Significantly, similar to the results from the primary mammary glands, the regenerated mammary glands by MaSCs from mice treated with the low dose of BPA showed increased duct density, secondary and tertiary branching, and a significantly greater number of hyperplastic lesions. Taken together, our study demonstrated that BPA exposure at very low dose could induce pre-neoplastic lesions in the mammary gland of pregnant mice, apparently by directly targeting MaSCs and implicates BPA as an exogenous endocrine factor that may promote pregnancy-associated mammary tumorigenesis.
Title: Long non-coding RNA H19 promotes cancer stemness and worsen breast cancer survival

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Body: Background: Cancer stem cells (CSC) are good sources of tumor initiation, heterogeneity, progression, and metastasis because of their unique characteristics. Several potential markers for CSCs have been suggested for breast cancer, including CD44\textsuperscript{+}/CD24\textsuperscript{−}/low, aldehyde dehydrogenase 1 (ALDH1), and epithelial cell adhesion molecule/epithelial-specific antigen. We previously reported that ALDH1 gene expression is related to aggressive phenotypes and poor prognosis in breast cancers. In this study, we conducted differential analysis of mRNA expression in ALDH1-positive breast cancer to identify genes associated with CSC. Next, we performed basic and clinical studies of one gene.

Methods: Messenger RNA was isolated from ALDH1-positive cells and ALDH1-negative cells in 5 ALDH1-positive breast cancers. Microarray analysis revealed that several genes were significantly associated with the ALDH1 gene. Among them, we examined a long non-coding RNA of H19 in this study. We evaluated the effect of H19 on CSCs using RNA interference and a sphere formation assay using two cell lines, HCC1937 and iCSCL10A cells. We also investigated H19 expression in 192 surgical specimens by \textit{in situ} hybridization and analyzed the relationship between H19 expression and clinic pathological findings in breast cancer patients.

Results: Through \textit{in vitro} experiments, we confirmed that suppression of H19 reduced sphere formation in both HCC1937 and iCSCL10A cells. Among surgical specimens, 48 samples (25\%) expressed H19. We verified thatH19 positivity was significantly higher in ALDH1-positive cases than in ALDH1-negative cases (68\% vs 9.7\%, p < 0.001). H19 was significantly highly expressed in triple-negative breast cancer (TNBC) (46\%) compared with other subtypes: luminal (33\%), luminal-HER2 (6\%), and HER2-enriched subtype (15\%). H19-positive patients showed significantly worse prognosis (5-year disease-free survival 75.8\% vs 91.5\%, p = 0.001 and 5-year overall survival 88.7\% vs 97.7\%, p = 0.002). The effect of H19 expression on prognosis was the most significant in TNBC compared to in other subtypes (5-year disease-free survival 63.6\% vs 88.9\%, p = 0.038).

Conclusions: H19 is clearly associated with CSCs and correlated with poor prognosis in breast cancer patients, particularly TNBC. Our future studies will investigate the role of H19 in maintaining the nature of CSCs and protein-coding genes associated with H19.
Rapamycin inhibits the stemness of mammary epithelial cells in the premalignant tissues of MMTV-ErbB2 transgenic mice

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Body: Rapamycin, a well-studied mTOR inhibitor, has been demonstrated to inhibit mammary carcinogenesis at multiple stages, including initiation, invasion, and metastasis, in preclinical animal models. Nevertheless, the cancer preventative potential and underlying mechanisms remain unclear, especially in individual breast cancer subtypes like ErbB2/Her2-positive breast cancers. ErbB2 amplification/overexpression is a particular clinical concern because it occurs in approximately one-third of human breast cancers and is associated with poor prognosis. Therefore, we used MMTV-ErbB2 transgenic mice as our model system to test the efficacy of rapamycin in the prevention of ErbB2-mediated mammary tumor development. Our initial data provided proof of concept regarding the anti-cancer effects of rapamycin in vivo. Indeed, rapamycin (1.5 mg/kg/day for 12 days) significantly reduced the volume and weight of syngeneic 78617 cell-derived mammary tumors in MMTV-ErbB2 mice, despite observed decreases in CD4⁺ and CD8⁺ immune cells. Since advanced mammary gland development can serve as an indicator of breast cancer risk, we investigated the effects of rapamycin on mammary gland development in MMTV-ErbB2 mice that were treated with low-dose rapamycin (1 mg/kg/day) between weeks 10 and 20 of age. As such, rapamycin significantly attenuated mammary morphogenesis at 20 weeks of age, as indicated by decreased branching density, ductal elongation, and proliferative index of the premalignant mammary glands. Flow cytometric analysis of isolated primary mammary epithelial cells (MECs) was performed using CD24 and CD49f markers to identify MEC populations. We found that rapamycin has a significant impact on MEC stemness based on changes in luminal (CD24⁹⁹CD49f̅), mammary stem cell (MaSC)-enriched (CD24⁹⁹CD49f̅), and myoepithelial/basal (CD24⁹⁹⁹⁹CD49f̅) MEC populations. We also used CD61 and CD49f markers to identify a population enriched with luminal progenitor cells (CD61⁹⁹⁹⁹CD49f̅) that was selectively inhibited by rapamycin. Consistent with our flow cytometric analyses, rapamycin inhibited the luminal progenitor cell-enriched population, self-renewal, and anchorage-independent cell growth of primary MECs, as demonstrated by colony-forming cell, mammosphere, and 3D culture assays, respectively. These functional stem cell assays further corroborate that rapamycin suppresses the stemness of primary MECs. Molecular analysis of MECs demonstrated that rapamycin inhibited mTOR signaling, as expected. Importantly, rapamycin also significantly suppressed the receptor tyrosine kinase/ErbB2, estrogen receptor, Wnt/β-catenin, and TGFβ/Smad3 signaling pathways prior to malignant transformation. Collectively, our study provides evidence that rapamycin has potential cancer preventative effects in the mammary glands of MMTV-ErbB2 mice during the premalignant risk window. These rapamycin-induced anti-cancer effects ultimately highlight the promising clinical significance of rapamycin for the prevention and treatment of human ErbB2-overexpressing breast cancers.
Title: Interactions between adipocyte stem cells and normal or tumoral mammary epithelial cells. Potential role of BRCA status and estrogen pathway

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**Body:** Prophylactic mastectomies are more and more frequent. As a result, breast reconstruction is also more frequent and mammary lipofilling often used to improve the aesthetic results. Contradictory clinical data have been published concerning the risk of breast cancer after using this technique. It is possible that Adipose-Derived Stem / Stromal Cells (ADSC or ASC), present in the lipofilling may interact with normal or tumoral epithelial mammary cells.

In this context, we studied the effect of conditioned media of human ASC on different tumor breast lines (MCF7, T47D, ZR75-1, SKBR3, SUM159). The ASCs were extracted from non-cancer patients with identical body mass index (BRCA1 and BRCA2 mutated or wild-type).

We observed that the conditioned media of ASC induced the proliferation of luminal, Her2 and basal-like tumor breast lines. This proliferative effect is more important when ASCs are differentiated and the BRCA mutation status has no impact on it. Finally, we have demonstrated an estrogen signaling within the ASCs which is set up during their differentiation. Differentiated ASCs express ER\(\alpha\) and produce 17\(\beta\)-estradiol (E2). This estrogenic pathway seems to be established independently of the BRCA mutational status. The proliferative effect on luminal mammary lines induced by conditioned media of ASC could pass through this estrogenic signaling, but remains to be confirmed.

These data highlight the fact that adipose-derived stem cells in mammary lipofilling should only be used after a cancer has been eliminated, as there are still some uncertainties concerning their implication on carcinogenesis.
Title: Targeting cancer stem-like cells metabolism via non-canonical notch signaling pathways in triple negative breast cancer

Fokhrul Hossain¹, Claudia Sorrentino¹, Ayse D Ucar Bilyeu¹, Margarite Matossian², Judy Crabtree¹, Antonio Pannuti¹, Matthew Burow², Todd Golde², Barbara Osborne³ and Lucio Miele¹. ¹LSUHSC, New Orleans, LA; ²University of Florida, Gainesville, FL; ³Tulane University, New Orleans, LA and ⁴University of Massachusetts Amherst, Amherst, MA.

Body: Triple negative breast cancer (TNBC) is a heterogeneous group of clinically aggressive diseases. TNBC patients have high risk of recurrence and metastasis, and current treatment options remain limited. Cancer stem-like cells (CSCs) have been linked to cancer initiation, progression and chemotherapy resistance. Therefore CSC-targeted therapies are keenly sought. There is strong evidence for the involvement of Notch signaling in TNBC. Notch1 is highly expressed in Basal-like 1 (BL1) and especially Mesenchymal-Stem-Like (MSL) TNBCs. Expression of Notch1 and its ligand Jagged1 correlate with poor prognosis. Moreover, strong evidence supports key roles of different Notch paralogs in breast CSCs. Here, we demonstrate that Notch activation by Jagged1-expressing stromal cells enhances transcription of the anti-apoptotic gene cIAP-2 (BIRC3), a known NF-κB target. This event is dependent on recruitment to the cIAP-2 promoter of NF-κB subunits, IKKα and Notch1. Short term exposure of MDA-MB-231 cells (MSL, PTEN wild-type), but not MDA-MB-468 cells (BL1, PTEN-null) to recombinant Jagged1 leads to AKT phosphorylation. This is suppressed by AKT inhibitors, IKK inhibitors, and dual mTORC1/2 inhibitors but not an mTORC1-selective inhibitor. These observations support a model where canonical and non-canonical mechanisms downstream of Notch1 trigger AKT phosphorylation and NF-κB activation in PTEN wild type TNBC cells. Rapid AKT phosphorylation downstream of Notch1 requires mTORC2, PI3K and IKKα, and contributes to NF-κB activation. This suggests a bidirectional crosstalk between the IKKα and AKT arms of this Jagged1-activated pathway. Importantly, we find co-localization of Notch1 with Mitochondria in MDA-MB-231 cells by confocal microscopy and Western blot of isolated mitochondrial fractions. We demonstrate that recombinant Jagged1 increases metabolism of TNBC cells. Knockdown of Notch1 or IKKα by siRNA decreases mitochondrial respiration and glycolysis. CSCs derived from MDA-MB-231 cells have increased Notch1, p-AKT, and oxidative metabolism compared to non-stem cells. AKT inhibition or IKK inhibition decreases both mitochondrial respiration and glycolysis of TNBC derived CSCs. Pharmacological inhibition of Notch cleavage by gamma secretase inhibitor (PF-03084014) in combination with AKT inhibitor (MK-2206) or IKK inhibitor (Bay11-7082) blocks CD90 hi or CD44+CD24 low sorted secondary mammospheres formation. Notably, we find similar results in TNBC patient derived xenograft (PDX) models. These data suggest that combination treatments affecting the intersection of Notch, NF-κB and AKT pathways have potential therapeutic importance in targeting CSCs in TNBC cases with high Notch1 expression.
**2017 San Antonio Breast Cancer Symposium**

**Publication Number:** P6-07-07

**Title:** Triple negative breast cancer classification according to cancer stem cell hypothesis

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**Body:**

**Background:**
Triple negative breast cancer (TNBC) is described by lack of ER and PR expression and HER2 overexpression. This subgroup has no targeted therapies and its prognosis is worse than other breast cancer subtypes. The Cancer Stem Cell hypothesis lies in two ideas; first, that breast cancers can initiate in different cell types, which can be any epithelial stem cells or any of their progeny, and, that this original cell will give the tumor a specific molecular profile. Our work proposes a division in molecular groups of TNBC looking for this tissue of origin molecular profile through gene expression data and probabilistic graphical models analyses.

**Material and methods:**
TNBC gene expression data was obtained from GSE31519 (n=494). 2000 most variable genes were selected for subsequent analysis. A functional network was built using a probabilistic graphical model approach. Functional nodes were defined, and its function was explored by Gene Ontology using DAVID. Then, a new molecular classification was generated using the activity of functional nodes and k-means. Subgroups were characterized and compared with previous TNBC molecular classifications.

**Results:**
Probabilistic graphical models defined a functional structure comprising 27 functional nodes. We found some Luminal, some Basal and a claudin-enriched node. Based on these nodes molecular subgroups were defined, following the cancer stem cell hypothesis. Thus, four subtypes: (Luminal Androgen receptor (LAR), basal, claudin-low and claudin-high) were defined matching tumor origin characteristics with those in the actual tumor sample. Tumors with low expression of claudins (CLDN-low subtype) had been differentiated in the first steps of the mammary epithelial development. Tumors with high expression of claudins (CLDN-High) had been originated at the second step of the development. Next step in the development are basal-epithelial cells, which will generate Basal subtype of TNBC. And finally, the last step in development is the differentiation to luminal cell, which is the origin of the Luminal subtype. Immune status, determined by immune functional nodes, showed prognostic value (p>0.05).

**Table 1**

<table>
<thead>
<tr>
<th>Cellular Classification</th>
<th>Tumor size</th>
<th>Grade</th>
<th>Nodal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>Rest</td>
<td>G3</td>
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<tr>
<td>Basal</td>
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<td>163</td>
<td>199</td>
</tr>
<tr>
<td>CLDN-High</td>
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<td>31</td>
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<tr>
<td>LAR</td>
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<td>54</td>
<td>29</td>
</tr>
<tr>
<td>Total</td>
<td>99</td>
<td>276</td>
<td>280</td>
</tr>
</tbody>
</table>

**Conclusion:**
Functional networks can provide a relevant molecular knowledge which complements the TNBC classification. From this
approach we establish a new classification taking into account the cancer stem cell hypothesis. Besides, this deep knowledge will allow a more accurate prediction of outcome and can also be used for diagnostic purposes and therapy selection.
Title: Dual TGFβ/BMP inhibition allows in vitro expansion of multiple cell types from normal and cancerous breast

Harikrishna Nakshatri¹, Manjushree Ananappa¹, Mayuri S Prasad¹, Brijesh Kumar¹, Yunlong Liu¹, Anna Maria Storniolo¹, Kathy D Miller¹ and Poornima Bhat-Nakshatri¹. ¹Indiana University School of Medicine, Indianapolis, IN.

Body: Functional modeling of breast epithelial hierarchy and stromal-epithelial cell interactions has been difficult due to inability to obtain sufficient stem-progenitor-mature epithelial cells and stromal cells. The recently developed epithelial reprogramming assay has partially overcome this limitation, allowing propagation of epithelial cells with stem, luminal progenitor and mature cell features. However, characterizing stromal cells using this assay is difficult because irradiated fibroblasts which can be difficult to distinguish from stromal cells are needed as feeder layer. A recent study demonstrated expansion of airway basal stem cells without a feeder layer through pharmacologic inhibition of TGFβ/BMP/SMAD signaling. We sought to develop this method for culture and expansion of cells from normal and cancerous breast samples. With appropriate modifications to growth media, we were able to obtain normal and stromal cells from breast biopsies of healthy women. The expanded cell population included CD10+/EpCAM- basal/myoepithelial cells, CD49f+/EpCAM+ luminal progenitor cells, CD49f-/EpCAM+ mature luminal cells, CD73+/EpCAM+/CD90- rare endogenous pluripotent somatic stem cells, CD73+/CD90+/EpCAM- mesenchymal stem cells, ALCAM (CD166)+/EpCAM+ cells, CD44+/CD24- cells, CD44+/CD24+ cells and ALDFLUOR+ stem/luminal progenitor cells. Epithelial cells were KRT14+, KRT19+ or both further documenting heterogeneity within epithelial cell population. We have extended this technique to grow breast epithelial cells from high-risk patients including BRCA1 mutant-carriers, tumor-adjacent normal and tumor cells from the same patient, pleural effusions and liver metastasis from breast cancer patients. Phenotypic characterization showed differences in the differentiation state of adjacent-normal and tumor cells. Tumor cells from pleural effusions showed remarkable phenotypic heterogeneity with a fraction of these cells expressing estrogen receptor. The assay described here, therefore, is versatile and provides resources to model epithelial-stromal interactions under normal and cancerous conditions as well as for genomics and screening of drugs to target metastasis on an individual level.

Susan G. Komen for the Cure and Department of Defense supported this work.
Title: Attenuation of progesterone driven mammary stem cell expansion by telapristone acetate

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Body: Purpose: The interplay of the ovarian hormones estradiol (E2) and progesterone (P4) contributes to the development of breast cancer, potentially aided by P4-induced expansion in mammary stem cells as observed in diestrous phase in mice, as well as the luteal phase and during pregnancy in women. Telapristone Acetate (TPA), a selective progesterone receptor modulator (SPRM), exhibits a protective effect against mammary carcinogenesis in rodents. TPA has been shown to display a more specific PR blockade and less toxicity when compared to RU486. We have examined the mammary stem cell pool expansion upon exposure to E2+P4 in mice and compared its attenuation by both TPA and RU486.

Methods: 8 week old female ovariectomized FVB mice weighing above 20g, were randomized into 4 treatment groups: sham (skin incision only, no pellets), E2+P4, E2+P4+Telapristone Acetate (TPA), E2P4+ Mifepristone (RU486). Eight experimental replicates were performed. At age 10 weeks, the mice were implanted with subcutaneous 30-day release pellets of E2 and P4 (0.3 mg E2 & 30.0 mg P4), E2P4+TPA (30.0 mg) and E2P4+RU486 (30.3 mg) in either flank. The mice were euthanized at day 15 of treatment. Single cell suspensions of the 4th inguinal mammary gland pair and one thoracic gland were prepared and labeled with cell surface markers. Lineage negative mammary gland cells were sorted into luminal and basal population subsets. The basal cell niche was identified as CD24+CD49fhi, the mammary stem cells (MaSC) within this niche are identified by CD61+CD49fhi. The cells were sorted on BD FACSAria 5-Laser and the data was analyzed using BD FACSDIVA. The D'Agostino-Pearson test was performed to determine the normal distribution and once normal distribution was confirmed one-way ANOVA (repeated measures) was performed to examine differences in percent cell populations with Tukey test for post-hoc analysis.

Results: The mammary stem cells (MaSC, CD61+CD49fhi) within the basal cell (CD24+CD49fhi) niche showed significant expansion at day 15 in mice implanted with Estrogen and Progesterone 30-day release pellet compared to sham (64.2%, 45.07% respectively; p=0.0392). This expansion was significantly attenuated in both TPA (-38.21%, p=0.011) and RU486 (-34.30%, p=0.002) treated mice compared to MaSC in mice treated with E2+P4 alone (+45.07%). Simultaneously, luminal progenitor cells (CD61+CD49f lo) show a marked reduction in E2+P4 treated mice compared to sham (17.77%, 45.54%, respectively; p=0.0375). Luminal mature cells (CD61-CD49f lo) show an expansion in E2+P4 treated mice compared to sham (82.23%, 54.41% respectively; %, p=0.0371). TPA significantly (58.40 %, p=0.061) suppresses LM cells expansion observed in the E2+P4 group. TPA and RU486 show significant suppression of the MaSC population in mouse mammary gland compared to the EP-treated mice.

Conclusion: TPA and RU486 alter the P4 driven changes in mammary gland cellular composition and in a manner consistent with the hypothesis that they will inhibit hormone-induced tumorigenesis in the mammary gland. To gain a better insight into this phenomenon, a high throughput transcriptomic profiling (RNASeq) of mammary stem cells isolated from the treatment groups is being performed.
Title: The ERβ4 variant induce transformation of the normal breast mammary epithelial cell line MCF-10A; the ERβ variants ERβ2, ERβ4 and ERβ5 increase aggressiveness of TNBC by regulation of hypoxic signaling

Michelle Faria¹, Samaneh Karami¹, Sergio Granados-Principal², Prasenjit Dey⁴, Akanksha Verma⁵, Dong S Choi⁶, Olivier Elemento⁵, Tasneem Bawa-Khalfe¹, Jenny C Chang⁶, Jan-Ake Gustafsson¹,⁷ and Anders M Strom¹. ¹University of Houston, Houston, TX; ²Hospital of Jaen, Jaen, Spain; ³University of Granada, Granada, Andalusia, Spain; ⁴The University of Texas MD Anderson Cancer Center, Houston, TX; ⁵Weill Cornell Medicine, New York, NY; ⁶Houston Methodist Hospital, Houston, TX and ⁷Karolinska Institutet, Stockholm, Sweden.

Body: Triple negative breast cancer (TNBC) still remains a challenge to treat in the clinic due to a lack of good targets for treatment. Although TNBC lacks expression of ERα, the expression of ERβ and its variants are detected quite frequently in this cancer type and can represent an avenue for treatment. We show that the variants of ERβ, namely ERβ1, ERβ2, ERβ4, and ERβ5, regulate aggressiveness of TNBC by regulating hypoxic signaling. RNA-seq of patient derived xenografts (PDX) from TNBC show expression of ERβ4 and ERβ5 variants in more than half of the samples. Furthermore, expression of ERβ4 in the immortalized, normal mammary epithelial cell line MCF-10A that is resistant to mammosphere formation caused transformation and development of mammospheres. By contrast, ERβ1, ERβ2 or ERβ5 were unable to support mammosphere formation. We have previously shown that all variants except ERβ1 stabilizes HIF-1α but only ERβ4 appear to have the ability to transform normal mammary epithelial cells, pointing towards a unique property of ERβ4. We propose that ERβ variants may be good diagnostic tools and also serve as novel targets for treatment of breast cancer.
2017 San Antonio Breast Cancer Symposium

Publication Number: P6-08-01

Title: Race and recurrence by PAM50 intrinsic subtype and ROR-PT score: The Carolina breast cancer study

Xuezheng Sun1, Katherine E Reeder-Hayes2, Erin L Kirk1, Linnea Olsson1, Chiu-Kit Tse1, Mary B Bell2, H Shelton Earp2, Lisa A Carey2, Charles M Perou2, Andrew F Olshan1 and Melissa A Troester1. 1University of North Carolina at Chapel Hill, Chapel Hill, NC and 2Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Body: Compared with white women, black women have higher breast cancer mortality. Racial differences have been reported in frequency of PAM50 intrinsic subtypes, and PAM50 intrinsic subtype is associated with prognosis. However, there is a paucity of data from large population-based studies with adequate representation of black women to evaluate its role in racial differences in breast cancer outcomes. We studied breast cancer recurrence in relation to PAM50 intrinsic subtype using data from the Carolina Breast Cancer Study Phase III (CBCS3), a large population-based prospective cohort study oversampled black women. The current analysis included 472 black and 463 white women with stage I-III tumor and available Nanostring gene expression data for PAM50 subtype and risk of recurrence score (ROR-PT, a score calculated based on subtype, proliferation level, and tumor size; categorized as low, medium, and high). Cox proportional hazard regression was used to estimate the association of recurrence with PAM50 intrinsic subtype and ROR-PT, overall and stratified by race, adjusted for tumor size, grade, and lymph node status.

During a median follow-up of 5.78 years (range=0.39-8.22), 110 recurrent cases were identified (68 blacks and 42 whites). Consistently with previous data, basal-like tumors had the highest risk for recurrence (reference=luminal A, adjusted hazard ratio [HR]=2.85, 95% confidence interval [CI]=1.44-5.66), followed by luminal B (adjusted HR=2.35, 95%CI=1.18-4.69) and Her2-enriched (adjusted HR=2.19, 95%CI=1.01-4.74). High-ROR-PT score was also associated with increased risk in both black (adjusted HR=1.55, 95%CI=0.93-2.60) and white (adjusted HR=2.07, 95%CI=1.04-4.11). Recurrence rate for black women with medium/low ROR-PT scores was suggested higher than that for white women with similar scores (adjusted HR=1.54, 95%CI=0.69-3.42).

Our results demonstrate that PAM50 subtype and ROR-PT score are associated with breast cancer recurrence in both black and white women. Given the higher relative frequency of poor prognosis subtypes in black women, these findings help explain prognosis disparities by race.
Primary neuroendocrine carcinoma of the breast – lessons learned from a ten year analysis of the National cancer data base (NCDB)

Claire E Rose¹, Robert E Heidel¹, John L Bell¹ and Amila Orucevic¹. ¹The University of Tennessee Graduate School of Medicine, Knoxville, TN.

Body: Primary neuroendocrine carcinomas of the breast (NECB) is rare (a reported incidence <5%). The significance of neuroendocrine differentiation and its impact on diagnosis, treatment and prognosis of NECB is controversial and based primarily on results from small retrospective case series reports. Our study objective is to define incidence, clinicopathologic characteristics, treatment patterns and prognosis of NECB and compare these to the most common invasive breast carcinoma (BC)-ductal (IDC).

A retrospective observational comparison study of NCDB patients (pts) from 2004 to 2014 compared all NECB pts with ICD-O-3 diagnosis codes 8246/3, 8041/3 and 8574/3 (well-differentiated neuroendocrine tumor, poorly differentiated/small cell carcinoma and invasive BC with neuroendocrine differentiation, respectively) to the same number of randomly selected IDC (8500/3) pts. Patients' clinicopathologic characteristics, treatment, and overall survival (OS) were analyzed using frequency statistics, chi-square, Kaplan-Meier and logistic regression.

1,790,023 pts had BC; 1,316,696=IDC (73.6%); 957=NECB (.0005%). NECB pts were significantly (p<.05) more likely to be: older, have larger tumors, grade 3 tumors, positive lymph nodes, ER, PR, HER2-negative tumors and higher TNM stage when compared to IDC. NECB pts were less likely to undergo surgery, radiation and anti-estrogen therapy. NECB pts had significantly worse 10-year OS than IDC pts (p<.001), with NECB pts being 3.4 times more likely to die in 10-years (95%CI 2.7-4.3).

Our study is the largest study to date on NECB (incidence 0.0005%) showing that NECB is aggressive and carries a significantly worse prognosis than IDC. Prospective randomized clinical trial(s) are unlikely, yet needed in order to conquer the current challenges of patients with NECB.

<table>
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<tr>
<th>Demographics</th>
<th>NECB</th>
<th>IDC (random sample)</th>
<th>Adjusted Odds Ratio *p&lt;.05</th>
<th>95%CI Lower</th>
<th>95%CI Upper</th>
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<td>N=957 N=957</td>
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<td>.2</td>
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<td>.9</td>
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<td>141 16.6</td>
<td>3.4*</td>
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<tr>
<td>Dead</td>
<td>515 59.5</td>
<td>710 83.4</td>
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</table>

R = Referent NS = Not significant
Body: Objective: Although neoadjuvant chemotherapy (NAC) has not been shown to improve survival compared with adjuvant therapy for patients with operable invasive breast cancer (IBC), it is often used to allow more limited surgery in the breast and axilla without compromising local control. We sought to evaluate national trends in mastectomy among patients with operable breast cancer treated with NAC and to characterize the contribution of demographic and tumor characteristics to changing trends.

Methods: We queried the National Cancer Database (NCDB) 2014 Participant User File for adult women who underwent surgery and received chemotherapy for unilateral T1-3N0-3M0 IBC diagnosed between 2010 and 2014. Surgery was classified as lumpectomy (BCS), unilateral mastectomy (UM) or bilateral mastectomy (BM). Molecular subtype was categorized according to ER, PR, and HER2 status. We used logistic regression to model surgery use (BM or UM vs BCS), adjusting for the following clinical covariates that were selected a priori: age, race/ethnicity, year of diagnosis, comorbidity score, metropolitan vs urban/rural residence, patient distance from treating facility, % with less than high school education (zip code based), insurance type, clinical stage, histology, and molecular subtype. As those who achieved pathologic complete response (pCR) after NAC should be ideal candidates for BCS, we also looked at this group separately. We then performed sensitivity analyses further controlling for region of the country and facility type, and for facility.

Results: We identified 235,339 patients who fulfilled our inclusion criteria. Of these patients, 25.3% were treated with NAC. Rates of pCR increased from 33.3% in 2010 to 46.3% in 2014 (p<0.001). Rates of BCS increased from 37.0% in 2010 to 40.8% in 2014 (p<0.001). While rates of UM decreased from 43.3% in 2010 to 34.7% in 2014 (p<0.001), rates of BM with or without reconstruction increased from 19.7% in 2010 to 24.6% in 2014 (p<0.001). Rates of BM without immediate reconstruction remained stable over time, from 11.8% in 2010 to 11.5% in 2014.

Among patients who received NAC, factors that were independently associated with both UM and BM (versus BCS) for both the entire cohort and those who achieved pCR included younger age, greater patient distance from facility, and higher clinical stage. Factors that were inversely associated with both UM and BM included black race and ductal histology. More recent year of diagnosis was inversely associated with UM and directly associated with BM. Asian race was associated with UM while non-Hispanic white race was associated with BM. Private or managed care insurance and higher area education were also associated with BM. These results were materially unchanged in sensitivity analyses.

Conclusion: Rates of pCR have increased over time among patients with operable IBC treated with NAC. While the rate of UM has declined over time, the rate of BM has increased. Significant sociodemographic differences exist between women who undergo BCS, and women who undergo UM and BM. Further study of factors that influence surgical decision-making in the NAC setting is warranted.
Title: United States real-world drug utilization patterns and associated overall survival in Medicare patients with newly-diagnosed metastatic triple negative breast cancer using surveillance, epidemiology, and end results-Medicare data

Abdalla Aly¹, Ruchit Shah¹, Kala Hill², Adrianus Reginald Waldeck² and Marc Botteman¹. ¹Pharmerit International, Bethesda, MD and ²Celldex Therapeutics, Hampton, NJ.

Body: Background: Limited information is available about treatment patterns for elderly patients (pts) with metastatic triple negative breast cancer (mTNBC). This analysis characterized real-world drug utilization patterns and associated overall survival (OS) for Medicare mTNBC pts.

Methods: Pts ≥66 years of age who were newly diagnosed with mTNBC between 2004 and 2011 were identified from the Surveillance, Epidemiology, and End Results (SEER)-Medicare linked database. Triple negative status was obtained from the SEER registry, except for HER2 that was unavailable from 2004-2009 during which we assumed that pts who had a claim for a HER2 test followed by absence of hormonal therapy to be presumed HER2 negative. Pts were followed from diagnosis to death, Medicare disenrollment, HMO enrollment, or 12/31/2013 (whichever occurred first) to characterize the sequence of chemotherapy received – first regimen (1R), second regimen (2R), and third regimen (3R) and median (interquartile, IQR) duration of and between regimens. OS estimates were reported using the Kaplan-Meier method.

Results: Among 694 mTNBC pts, 69 died within 30 days of diagnosis and were excluded. In the remaining 625 pts observed from 2004 through 2013 (median age: 75 years; Charlson comorbidity index (CCI) ≥2: 21%; and median follow-up: 11.4 months), 317 (51%) received chemotherapy. Of the 317 pts, 161 got only 1R, 88 got only 2R, and 68 got 3R+. Compared to pts on 1R, pts on 2R were significantly younger (median age: 2R, 72; 1R, 75 years), married (2R, 40%; 1R, 30%), had fewer comorbidities (CCI≥2: 2R, 16%; 1R, 23%). The top 2 most commonly prescribed single agents in 1R, 2R, and 3R were: 1R, paclitaxel followed by capecitabine; 2R, capecitabine followed by paclitaxel; 3R, gemcitabine followed by capecitabine. The most common combination regimen given was taxane-based in 1R (57%) and 2R (70%).

<table>
<thead>
<tr>
<th>Drug utilization patterns</th>
<th>First Regimen (n=317)</th>
<th>Second Regimen (n=156)</th>
<th>Third Regimen (n=68)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Agent</td>
<td>205 (65%)</td>
<td>74 (47%)</td>
<td>40 (59%)</td>
</tr>
<tr>
<td>Microtubule inhibitors</td>
<td>80 (39%)</td>
<td>23 (31%)</td>
<td>NR</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>72 (35%)</td>
<td>18 (24%)</td>
<td>NR</td>
</tr>
<tr>
<td>Anthracyclines</td>
<td>29 (14%)</td>
<td>13 (18%)</td>
<td>NR</td>
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<tr>
<td>Doxorubicin</td>
<td>27 (13%)</td>
<td>13 (18%)</td>
<td>NR</td>
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<tr>
<td>Antimetabolites/Others</td>
<td>96 (47%)</td>
<td>38 (51%)</td>
<td>27 (73%)</td>
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<tr>
<td>Capecitabine</td>
<td>51 (25%)</td>
<td>20 (27%)</td>
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<tr>
<td>Gemcitabine</td>
<td>NR</td>
<td>NR</td>
<td>13 (35%)</td>
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<tr>
<td>Others</td>
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<td>NR</td>
<td>NR</td>
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<tr>
<td>Combination Regimens</td>
<td>112 (35%)</td>
<td>82 (53%)</td>
<td>28 (41%)</td>
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<tr>
<td>Taxane-based</td>
<td>64 (57%)</td>
<td>57 (70%)</td>
<td>NR</td>
</tr>
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</table>

NR, not reported per DUA with NCI. %, col. % relative to single/comb. category

Median (IQR) durations of 1R, 2R, and 3R were 2.7 (1.4-4.4), 3.1 (1.6-5.0), and 2.3 (0.9-4.5) months, respectively. Median time from diagnosis to start of 1R was 1.6 (0.9-2.8) months. Median time to start of 2R and 3R after the end of the previous regimen was 4.6 (2.1-8.1), and 6.2 (3.3-11.0) months, respectively. The median (12-month) OS was 7 months (34%) for all pts and ranged from 3.5 (17%) in the untreated to 25.3 (88%) months in 3R+ pts.

Conclusions: About half of Medicare mTNBC pts do not receive chemotherapy in the real world. Paclitaxel and capecitabine were the most commonly used single agents and taxane-based combination therapy was the most commonly used combination.
Title: Association of body mass index (BMI) with chemotherapy administration and emergency room (ER) visits among breast cancer patients

Sharon H Giordano¹, Jiangong Niu¹, Hui Zhao¹, Daria Zorzi¹ and Mariana Chavez-Mac Gregor¹. ¹The University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Background: Obese patients may be more likely to have treatment-related complications. Little is known about the association between BMI and chemotherapy-related ER visits and hospitalizations among breast cancer patients. In this study we evaluated the association between BMI and ER visits/hospitalizations in a large sample of breast cancer patients. Methods: We identified beneficiaries from the commercial MarketScan Health Risk Assessment database with breast cancer-specific claims between 2009-2014. All patients underwent mastectomy or lumpectomy and had BMI data available. BMI was categorized as normal (<25), overweight (≥25 and <30) and obese (≥30). Descriptive statistics were used to compare patient and treatment variables by BMI. Chemotherapy-related ER visits/hospitalizations in the 6 months after diagnosis were identified. Cox regression models were used to identify factors associated with ER visits and hospitalizations. Results: Among 7,830 patients included, 33.2% were classified as normal/underweight, 29.5% as overweight and 37.2% as obese. 2,928 (37%) patients were treated with chemotherapy. BMI differed significantly according to age, geographic region, comorbidity score, and treatment received. Among patients not treated with chemotherapy, the rates of ER visits/hospitalizations were: 11.4% for normal patients, 13.7% for overweight and 15.2% for obese patients (p=0.004). For chemotherapy-treated patients, the rates of ER visit/hospitalization were: 22.6% for normal patients, 24.3% for overweight and 30.2% for obese patients (p<0.001). In the multivariable model, chemotherapy-treated patients with obesity had higher risk to have ER visits/hospitalizations (HR=1.37, 95%CI 1.14–1.64) than patients with normal BMI. No significant increase was seen among overweight patients (OR=1.09; 95%CI 0.90-1.33). Conclusions: In this cohort of patients, obesity was associated with increased risk of ER visits/hospitalizations, suggesting that careful follow-up is warranted in these patients, particularly among those receiving chemotherapy.
Decline in compliance to breast cancer screening in France: Results of the 5th EDIFICE survey

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Body: Background
The EDIFICE surveys have been conducted since 2005 to provide a better understanding of the participation of the French population in cancer screening programs. The breast cancer (BC) screening program is nowadays widely implemented throughout the target female population: women aged 50-74 years are invited for a mammogram once every two years. We analyzed the behavior of women over time (between 2005 and 2016) regarding BC screening according to age-group, socioprofessional categories (SPC) and social vulnerability.

Methods
The fifth nationwide observational survey, EDIFICE 5, was conducted by phone interviews using the quota method. A representative sample of 1299 subjects aged 50-74 years with no history of cancer was interviewed between November 22 and December 7, 2016. Interviewees (women aged 50-74 yrs; N=657) were asked about their uptake of BC screening, and the date of the last examination to determine the follow-up rate. Demographic data on social characteristics and SPC were also collected to determine the level of social vulnerability (validated EPICES score).

Results
Compliance with BC screening (a mammogram within the past 2 years) decreased significantly from 81% in 2014 to 75% in 2016 ($P=0.02$). From 2014, a significant impact of age was observed ($P=0.02$ in 2014 and 2016). In 2016, and for the first time, a significant and disturbing decrease in follow-up rate was recorded among younger women (age 50-54 yrs: 88% in 2014 vs 74% in 2016, $P=0.01$). SPC had no effect on compliance with screening. However, since 2008, fluctuations have been observed among unemployed individuals (U), with a significant decline in compliance in 2016 (U: 81% in 2014 vs 73% in 2016, $P=0.03$). In 2016, social vulnerability was seen as a factor that negatively impacts on compliance with BC screening ($P<0.01$), but not in 2011 or 2014. We also observed a significant overall decrease in 2016 among both vulnerable (76% in 2014 vs 65% in 2016; $P=0.04$) and non-vulnerable populations (85% in 2014 vs 79% in 2016, $P=0.04$), highlighting a general loss of confidence.

Conclusion
For the first time since 2005, compliance with BC screening dropped significantly in France in 2016. A significant overall decline was observed regardless of social vulnerability status. Disturbing significant decreases were observed among the younger age-group (women aged 50-54 yrs) of the target population of the organized program, and among unemployed women. Although organized programs have been shown to ensure equitable access to cancer screening, this achievement remains precarious and requires regular monitoring.
INTRODUCTION: Women with triple-negative breast cancers (TNBC) have a tendency to present at younger ages and with more advanced disease. We sought to comprehensively evaluate the characteristic features, surgical management, and survival outcomes of a large, population-based cohort of patients with TNBC according to age at diagnosis.

METHODS: We queried the Surveillance, Epidemiology, and End Results (SEER) database to identify women aged 18 years or older with a diagnosis of TNBC between 2010-2014. Clinicopathologic and treatment level variables were compared amongst TNBC patients according to age at the time of TNBC diagnosis. The Kaplan-Meier method and Cox PH Regression was then used to examine short-term breast cancer-specific survival (BCSS) outcomes.

RESULTS: Between 2010-2014, 214,138 women were diagnosed with breast cancer, of which 23,614 (11.13%) had TNBC. The median age at TNBC diagnosis was 57.7 years. Younger TNBC patients were more likely to be of African American (<40 years, 20.1% vs. ≥70 years, 15.5%; p<0.001) or Hispanic race (<40 years, 21.9% vs. ≥70 years, 7.0%; p<0.001), diagnosed with larger tumors (T2-T3; <40 years, 70.2%; 40-49 years, 61.7%; 50-59 years, 55%; 60-69 years, 48.1%; ≥70 years, 49.5%; p<0.001) and present with lymph node positive disease (<40 years, 36.7%; 40-49 years, 34.8%; 50-59 years, 32.5%; 60-69 years, 27.5%; ≥70 years, 27.9%; p<0.001). With respect to local therapy, younger women also had a greater tendency to undergo bilateral mastectomy (<40 years, 34.3%; 40-49 years, 23.1%; 50-59 years, 13.4%; 60-69 years, 8.4%; ≥70 years, 3.3%; p<0.001). The estimated one and four-year BCSS for the entire cohort was 94.4% and 79.7%, respectively, with the youngest women <40 years and older women ≥70 years demonstrating the poorest unadjusted BCSS at four years (<40 years, 76.95%; 40-49 years, 82.1%; 50-59 years 80.9%; 60-69 years 81.7%; ≥70 years, 78.6%; log rank p<0.001). In Cox PH analysis adjusting for race, stage, pathologic features, and local therapy, age greater than 70 years remained significantly associated with worse cancer-specific survival (HR 1.60, 95% CI 1.39-1.84).

CONCLUSION: In the population studied, more than 40% of very young women with TNBC are of African American or Hispanic race. When compared to older ages, younger women with TNBC are more likely to receive bilateral mastectomy and have more advanced stage at presentation. Women at both age extremes (≥70 years and <40 years at diagnosis) demonstrate worse cancer-specific survival outcomes. In older women, this may be due to undertreatment, and in younger women, to delays in diagnosis and/or worse tumor biology. Further studies are needed to evaluate age-related discrepancies in local and systemic therapy and cancer-specific survival in TNBC.
Title: Incidence and risk factors for congestive heart failure in early breast cancer received anthracyline and/or trastuzumab; big-data analysis of Korean health insurance review and assessment service database

Body: Background: Anthracycline (AC) and/or trastuzumab (T) are the most commonly used for neo-/adjuvant therapy for early breast cancer. However, use of those regimens are limited owing to congestive heart failure (CHF). Although reported incidence from pivotal trials is very low and acceptable, no big data-based population study has not been conducted in Koreans yet. The aim of this study was to analyze the incidence, time to occurrence, and risk factors for CHF in patients with early breast cancer, who were treated with AC and/or T therapy, in Korea.

Methods: We used the Health Insurance Review and Assessment Service database and included women with no prior history of CHF who were aged >19 years and diagnosed as having early breast cancer between 2007 and 2016. Only patients who had received breast cancer surgery and AC and/or T therapy were included. Patients with metastatic cancer codes were excluded. Result: In total, 86,086 patients were included for this analysis. The incidence and median time to occurrence of CHF according to chemotherapy type were, 3.27% and 683.5 days in the AC only group, 6.39% and 374 days in the AC followed by T group, and 4.43% and 286 days in the T with or without non-AC group, respectively.

The multivariate Cox regression analysis revealed that the adjusted hazard ratio (HR) for CHF was increased with older age; in those aged ≥65 years versus <50 years (HR, 2.79; 95% confidence interval [CI], 2.50–3.12). The HR in the AC followed by T group was significantly higher than that in the AC only group (HR, 2.21; 95% CI, 2.05-2.37). The T with or without non-AC group also showed a significantly higher HR than the AC only group (HR, 1.67; 95% CI, 1.37-2.04). CCI scores of ≥2 were significant predictors of CHF; score 2 versus 0 (HR, 1.30; 95% CI, 1.18-1.45), and score ≥3 versus 0 (HR, 1.87; 95% CI, 1.69-2.06). In addition, preexisting medical conditions were significant predictors for CHF: hypertension (HR, 1.58; 95% CI, 1.45-1.72), diabetes (HR, 1.17; 95% CI, 1.07-1.28), and ischemic heart disease (HR, 1.60; 95% CI, 1.45-1.76).

Conclusion: This is the first big data-based population study in Korea on the development of CHF after treatment with AC and/or T. The overall incidence of CHF was 3% to 6%, with a median time to occurrence of 1 to 2 years. Adjusted HR increased with older age, AC followed by T therapy, CCI scores ≥2, and preexisting conditions.

Table 1. Incidence and median time to occurrence of congestive heart failure according to chemotherapy type

<table>
<thead>
<tr>
<th>Chemotherapy type</th>
<th>Total</th>
<th>CHF event (%)</th>
<th>Median time to occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC only</td>
<td>66,699</td>
<td>2,182 (3.27%)</td>
<td>683.5</td>
</tr>
<tr>
<td>AC followed by T</td>
<td>17,062</td>
<td>1,090 (6.39%)</td>
<td>374</td>
</tr>
<tr>
<td>T ± non-AC</td>
<td>2,325</td>
<td>103 (4.43%)</td>
<td>286</td>
</tr>
</tbody>
</table>

Table 2. Cox proportional hazards model for congestive heart failure, adjusted for age, chemotherapy type, and Charlson comorbidity index score

<table>
<thead>
<tr>
<th>Variable</th>
<th>P-value</th>
<th>HR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (ref &lt;50 years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age Group</td>
<td>p-value</td>
<td>Hazard Ratio</td>
<td>95% CI</td>
</tr>
<tr>
<td>-----------------</td>
<td>---------</td>
<td>--------------</td>
<td>----------</td>
</tr>
<tr>
<td>50-64 years</td>
<td>&lt;0.0001</td>
<td>1.54</td>
<td>1.43-1.66</td>
</tr>
<tr>
<td>≥65 years</td>
<td>&lt;0.0001</td>
<td>2.79</td>
<td>2.50-3.12</td>
</tr>
<tr>
<td>Chemotherapy type (ref: AC only)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC followed by T</td>
<td>&lt;0.0001</td>
<td>2.21</td>
<td>2.05-2.37</td>
</tr>
<tr>
<td>T ± non-AC</td>
<td>&lt;0.0001</td>
<td>1.67</td>
<td>1.37-2.04</td>
</tr>
<tr>
<td>Charlson comorbidity index score (ref: 0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.1206</td>
<td>1.08</td>
<td>0.98-1.19</td>
</tr>
<tr>
<td>≥2</td>
<td>&lt;0.0001</td>
<td>1.30</td>
<td>1.18-1.45</td>
</tr>
<tr>
<td>≥3</td>
<td>&lt;0.0001</td>
<td>1.87</td>
<td>1.69-2.06</td>
</tr>
</tbody>
</table>
Title: Impact of age at diagnosis of metastatic breast cancer on overall survival in the real-life "ESME" cohort

Sophie Frank¹, Corinne Tchokothe¹, Matthieu Carton¹, Emmanuelle Mouret-Fourme¹, Coralie Dubot¹, Mario Campone², Barbara Pistilli³, Florence Dalenc⁴, Audrey Mailliez⁵, Christelle Levy⁶, Véronique D'Hondt⁷, Marc Debled⁸, Marianne Leheurteur⁹, Bruno Coudert¹⁰, Christophe Perrin¹¹, Anthony Gonçalves¹², Lionel Uwer¹³, Jean-Marc Ferrero¹⁴, Jean-Christophe Eymard¹⁵, Thierry Petit¹⁶, Marie-Ange Mouret-Reynier¹⁷, Tahar Guesmia¹⁸, Thomas Bachelot¹⁹, Paul Cottu¹. ¹Institut Curie, Paris/Saint Cloud, France; ²Institut de Cancérologie de l'Ouest - Paul Papin, Angers, France; ³Institut Gustave Roussy, Villejuif, France; ⁴Institut Claudius Régaud, Toulouse, France; ⁵Centre Oscar Lambret, Lille, France; ⁶Centre François Baclesse, Caen, France; ⁷Institut du Cancer de Montpellier, Montpellier, France; ⁸Institut Bergonié, Bordeaux, France; ⁹Centre Henri Becquerel, Rouen, France; ¹⁰Centre Georges-François Leclerc, Dijon, France; ¹¹Centre Eugène Marquis, Rennes, France; ¹²Institut Paoli-Calmettes, Marseille, France; ¹³Institut de Cancérologie de Lorraine-Alexis Vautrin, Vandœuvre-lès-Nancy, France; ¹⁴Centre Antoine Lacassagne, Nice, France; ¹⁵Institut Jean Godinot, Reims, France; ¹⁶Centre Paul Strauss, Strasbourg, France; ¹⁷Centre Jean Perrin, Clermont-Ferrand, France; ¹⁸R&D Unicancer, France and ¹⁹Centre Léon Bérard, Lyon, France.

Body: Background

Young age is a known poor prognosis factor in early stage breast cancer (BC). Its value is less documented for metastatic BC (MBC). Guidelines state that age should not guide the treatment strategy. We used the ESME database to evaluate the impact of age at MBC diagnosis on overall survival (OS).

Patients and Methods

ESME is a unique national cohort, collecting retrospective data using clinical trial-like methodology. It included all consecutive MBC patients (pts) who initiated at least 1 treatment in one of the 18 participating French cancer centers between 01/01/2008 and 12/31/2014. The database was locked on 12/8/2016. Primary objective were the comparisons of MBC characteristics between age groups (<40, 40 to 60 and >60 years (y)) and the evaluation of the impact of age at MBC diagnosis on OS. Interaction between age and tumor subtype was tested using a Cox regression model.

Results

Among 16 703 included pts, 1539 had no information on tumor receptors (ER/PR/HER2) and 682 had an exclusion criteria (unknown age, men or other cancer in the last 5y), leaving 14 482 for analysis. At the onset of MBC, 902 pts (6.2%), 6269 (43.3%) and 7311 (50.5%) were <40y, 40y to 60y and older than 60y respectively. Median follow-up was 54.8 months.

Pts <40 had significantly more aggressive presentations than other age groups: more HER2+ (26.5%), and triple negative (26.4%) subtypes, more visceral involvement (57.1%), and shorter time to metastasis (26.9% between 6 to 24 months) (all p-value vs other age groups <0.0001).

MBC characteristics according to age groups

<table>
<thead>
<tr>
<th>Age at MBC diagnosis (years)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;40</td>
<td>40-60</td>
</tr>
<tr>
<td>Tunor subtype</td>
<td></td>
</tr>
<tr>
<td>HR+/HER2-</td>
<td>425 (47.12)</td>
</tr>
<tr>
<td>HR-/HER2-</td>
<td>238 (26.39)</td>
</tr>
<tr>
<td>HER2+</td>
<td>239 (26.5)</td>
</tr>
<tr>
<td>Type of metastasis, N(%)</td>
<td></td>
</tr>
<tr>
<td>Bone only</td>
<td>219 (24.31)</td>
</tr>
<tr>
<td>Non visceral</td>
<td>168 (18.65)</td>
</tr>
<tr>
<td>Visceral</td>
<td>514 (57.05)</td>
</tr>
<tr>
<td>Time to first metastasis (months), N(%)</td>
<td></td>
</tr>
<tr>
<td>&lt; 6</td>
<td>304 (33.74)</td>
</tr>
</tbody>
</table>
Overall, median OS was identical in the different age groups: 39.1, 41.1 and 39.8 months for pts <40, 40-60 and >60, respectively (p=0.2). Tumor subtype and age showed a significant interaction on OS (p<0.0001), especially among HER2+ MBC.

### Overall survival (months) according to tumor subtypes and age groups

<table>
<thead>
<tr>
<th>Tumor subtype</th>
<th>Age groups (years)</th>
<th>p-value (log-rank)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;40</td>
<td>40-60</td>
</tr>
<tr>
<td>HR+/HER2-</td>
<td>46.4 (CI 95% 40.5-55.4)</td>
<td>47.8 (CI 95% 46-50)</td>
</tr>
<tr>
<td>HER2+</td>
<td>60.7 (CI 95% 45.6-76.4)</td>
<td>50.4 (CI 95% 46.3-56.3)</td>
</tr>
<tr>
<td>Triple negative</td>
<td>14 (CI 95% 11.5-16.5)</td>
<td>14.7 (CI 95% 13.7-15.9)</td>
</tr>
</tbody>
</table>

Anti-HER2 with first-line treatment was given preferentially to young pts: 86.6, 81.9 and 74.9% for pts <40, 40-60 and >60, respectively (p<0.0001).

**Conclusion**

At onset of MBC, young age was associated with more aggressive presentations, however with no global impact on OS. Pts <40 with HER2+ disease carried a better prognosis, maybe related to therapy.
Title: Regional variation in de novo metastatic breast cancer survival improvement over time using an institutional registry to support SEER analysis: 1990-2010

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Body: Background: It is not known how much de novo MBC (dnMBC) survival varies by where one lives and receives treatment in the US. Variation could exist depending on access to care, insurance availability, geographic area, and treatment options (urban vs. rural). Our objective is to measure variance in survival by Surveillance, Epidemiology, and End Results (SEER) region and compared to a non-academic institutional cohort.

Methods: We compared change in disease specific survival (DSS) over time intervals 1990-1998, 1999-2004 and 2005-2010, following diagnosis of first primary dnMBC among women in the SEER 9 population-based cancer registry using SEER 9 without Seattle-Puget Sound (S-PS) (n = 11,139) and Seattle Puget Sound separately (n = 1787). In a separate analysis we calculated and compared dnMBC DSS in our community-based cancer center registry located in the Seattle-Puget Sound region (n = 247) for the same time intervals. For the institutional cohort time to event used the outcome death from breast cancer confirmed from the patient chart or death certificate if information on cause of death was not available in the chart. For SEER data we used the SEERstat calculation for cause-specific survival as equivalent for disease specific survival (DSS). We estimated 5-year DSS cumulative incidence with 95% confidence intervals using the Kaplan-Meier method and compared survivor function equality by diagnosis years with log-rank tests. SEER*stat 8.3.4 was used for the SEER regional comparison and SPSS 24 for the institutional.

Results: Patient age in the SEER registry population ranged from 18-93 years. The institutional cohort patient age range was 24-94 years. DSS improved over time for SEER 9 without Seattle-Puget Sound [1990-1998: 18.8% (95% CI: 17.6%, 20.0%); 1999-2004: 22.0% (95% CI: 20.5%, 23.4%); 2005-2010: 24.6% (95% CI: 23.2%, 26.0%) (log-rank test=61.59, p<0.001)]. DSS for SEER Seattle-Puget Sound had a similar significant improvement gradient over time [1990-1998: 20.1% (95% CI: 17.1%, 23.2%); 1999-2004: 25.6% (95% CI: 21.9%, 29.4%); 2005-2010: 33.4% (95% CI: 29.7%, 37.1%) (log-rank test=42.46, p<0.001)]. DSS was significantly better in the Seattle-Puget Sound region in 2005-2010 (33.4%) compared to SEER9 without Seattle-Puget Sound (24.6%) (p=.017). Among dnMBC cases at the non-academic community cancer center, five year dnMBC DSS improved over time as well and by a larger margin [1990-1998: 28%, 95% CI: 18.2%, 37.8%; 1999-2004: 48%, 95% CI: 33.9%, 58.9%; 2005-2010: 55%, 95% CI: 45.3%, 64.5% (log rank test=9.65, p=.008)].

Conclusions: The SEER regional comparison indicates a significant regional survival difference for breast cancer patients with de novo stage IV metastatic breast cancer. Better survival in the Seattle-Puget Sound region is supported by the retrospective cohort analysis results from a center with a more detailed registry and complete follow up in the same region. Supplementation of regional survival SEER analysis with detailed analysis from an embedded institutions’ dedicated registry could be used to enhance evaluation of factors such as standard of care that impact survival improvement.
Title: Understanding the etiology of osteopenia and osteoporosis in young breast cancer survivors compared to cancer-free women

Cody A Ramin¹, Betty J May¹, Richard BS Roden²,³, Michelle McCullough¹, Deborah K Armstrong²,³ and Kala Visvanathan¹,²,³.
¹Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; ²Johns Hopkins Kimmel Cancer Center, Baltimore, MD and ³The Johns Hopkins School of Medicine, Baltimore, MD.

Body: Background: Our group previously reported that young breast cancer (BC) survivors have a higher risk of osteopenia/osteoporosis compared to their cancer-free peers. In order to develop successful interventions we need to understand the major contributing factors. Therefore, we investigated bone loss in young BC survivors by age at diagnosis, tumor characteristics and BC treatment compared to their cancer-free peers.

Methods: We studied 775 women (211 BC survivors, 564 cancer-free) with familial risk of breast and/or ovarian cancer in the Breast and Ovarian Surveillance Service (BOSS) cohort at Johns Hopkins. Survivors were diagnosed with stage 0-III BC <5 years prior to enrollment. The comparison group was cancer-free women at enrollment. Osteopenia and osteoporosis were ascertained based on self-reported physician diagnosis in baseline and follow-up questionnaires. Prevalent cases of osteopenia or osteoporosis were excluded. Multivariable (MV)-adjusted Cox proportional hazard models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) of osteopenia and/or osteoporosis among BC survivors compared to cancer-free women. BC survivors were stratified by age at diagnosis, estrogen-receptor (ER) tumor status, and BC treatment. MV models were adjusted for age, menopausal status, body mass index, physical activity, smoking, alcohol use, hormone replacement therapy and early oophorectomy.

Results: Mean time from BC diagnosis to enrollment was 1.4 years for survivors and mean age at BC diagnosis was 47 years. At baseline, BC survivors were more likely to be slightly older, postmenopausal, and current vitamin D users and less likely to have had an early bilateral oophorectomy compared to cancer-free women. During a mean follow-up time of 5.7 years, 66% of BC survivors and 54% of cancer-free women reported having ≥1 bone density exam and 112 incident cases of osteopenia/osteoporosis were identified (75% osteopenia only). BC survivors diagnosed at age ≤50 years had a 2-fold increased risk of osteopenia/osteoporosis compared to cancer-free women (HR=2.05, 95% CI=1.24-3.40). Risk of bone loss was similar among survivors with ER-positive tumors compared to cancer-free women (HR=2.04, 95% CI=1.30-3.22). No association was observed for BC survivors treated with tamoxifen only or chemotherapy only. BC survivors treated with aromatase inhibitors (AIs) only had almost 3-fold increased risk of osteopenia/osteoporosis compared to cancer-free women (HR=2.92, 95% CI=1.38-6.17). BC survivors treated with chemotherapy + tamoxifen and chemotherapy + AIs had over 2- and 4-fold increased risk of osteopenia/osteoporosis compared to cancer-free women (HR=2.28, 95% CI=1.04-5.00; HR=4.09, 95% CI=1.99-8.42, respectively). Results suggest that risk of bone loss was highest within 5 years after BC diagnosis.

Conclusion: Our results demonstrate that osteopenia/osteoporosis incidence is higher in BC survivors compared to cancer-free women and risk varies by age at diagnosis, ER-status and BC treatment. Our findings provide support for a baseline evaluation of bone density close to diagnosis in BC survivors with familial risk. Future studies are needed to address the frequency of monitoring among specific age and treatment groups.
Title: Statins and breast cancer recurrence: A population-based case-control study

Meredith L Vandermeer¹, Melanie C Francisco¹, Kathryn E Richert-Boe¹, Charisma L Jenkins¹ and Sheila Weinmann¹. ¹Kaiser Permanente Northwest, Center for Health Research, Portland, OR.

Body: Background: Breast cancer is the most common cancer among women and a significant number of women experience recurrence. Statins, drugs for lowering cholesterol, were introduced in 1987, and by 2011-12, 27.9% of U.S. adults over the age of 40 reported using cholesterol-lowering medication. Statins may impact other diseases, beyond cardiovascular disease, including cancer. Statin use and breast cancer recurrence or disease-free survival has previously been explored in 8 cohort studies and 1 case-control study with mixed results.

Methods: We designed a nested case-control study with Kaiser Permanente members in NW Oregon and SW Washington to examine the association between statins and breast cancer recurrence.

All subjects were women diagnosed with invasive breast cancer from 1980-2010. Subjects were KPNW health plan members at diagnosis, had local or regional stage cancer, were ER and/or PR positive, and were treated with tamoxifen for 180 days or more. Cases had breast cancer recurrence validated by medical record review. Controls were matched on race, SEER stage, age at diagnosis, year of diagnosis and pattern of health plan membership, and were recurrence-free for at least 12 months longer than their matched case (up to 3:1 match). The index date was the recurrence date for the case and the date for an equivalent period after diagnosis for the matched control. We collected data from medical records and from pharmacy, laboratory, tumor registry, and membership health plan databases.

We performed bivariate analysis to look at characteristics associated with recurrence. A priori, we identified potential confounding variables based on literature review and clinical knowledge. Using multivariable logistic regression analysis, we assessed statin use in relation to breast cancer recurrence, accounting for factors that may alter the association.

Results: We identified 306 cases with breast cancer recurrence and 679 matched controls. Thirty-five cases (11.4%) and 67 controls (9.9%) were prescribed statins at any time between their breast cancer diagnosis and index date. Nearly everyone on statins was prescribed lipophilic statins (99%). We calculated dose equivalents for all statins, using 20 mg of simvastatin as one dose. Among those who took statins, the average number of equivalent doses per day after diagnosis was 1.20 (1.19 for cases; 1.21 for controls) and the average duration of taking statins between diagnosis and recurrence was 2.65 years (2.75 for cases; 2.59 for controls). In our preliminary conditional analysis, we found that post-diagnostic statin use was not associated with a decreased odds of breast cancer recurrence (OR 1.37, 95% CI: 0.79-2.36) after adjusting for age, year of diagnosis, race, BMI, menopause status, tamoxifen use, type of surgery, treatment, smoking history, Charlson score, AJCC summary stage, and Nottingham grade.

Conclusions: While other studies have reported that statins may be associated with decreased odds of breast cancer recurrence, our preliminary multivariable analyses that looked at any statin use between diagnosis and index date do not support those results.
Title: Breast cancer risk in chronic users of phthalate-containing medications: A Danish nationwide cohort study

Thomas P Ahern¹, Deirdre P Cronin-Fenton², Anne Broe³, Sinna Pilgaard Ulrichsen², Bernard F Cole¹, Timothy L Lash⁴, Henrik Toft Sørensen², Rulla M Tamimi⁵ and Per Damkier³. ¹University of Vermont, Burlington, VT; ²Aarhus University, Aarhus, Denmark; ³University of Southern Denmark, Odense, Denmark; ⁴Emory University, Atlanta, GA and ⁵Brigham and Women's Hospital/Harvard Medical School, Boston, MA.

Body: Background. Phthalates are ubiquitous in consumer goods (e.g., food containers, cosmetics, and pharmaceuticals), from which they readily leach into the environment. Phthalates interfere with hormonal signaling and may affect reproductive, developmental, and cancer endpoints. Preclinical evidence implicates some phthalates in breast cancer progression—particularly dibutyl phthalate (DBP), which potentiates the estrogen receptor (ER). Associations between phthalates and breast cancer incidence have not been thoroughly investigated. Users of phthalate-containing medications have up to 70-fold higher urinary phthalate levels than other individuals, and represent a highly exposed population for efficient study of phthalate health effects.

Methods. We used the Danish Drug Information Database to identify all phthalate-containing oral medications marketed during the study period. We recorded the product code and the type and mass of phthalate per pill. We identified a nationwide cohort of women at risk for a first cancer between 2005—2015, and who had no previous exposure to a phthalate-containing drug. Using the National Prescription Registry we characterized time-varying, medication-borne phthalate exposure. Incident cancers were ascertained by linking to the Danish Cancer Registry. We fit Cox regression models to estimate associations between cumulative phthalate exposures and breast cancer incidence. Exposures were updated annually and lagged by 1 year. We adjusted for established risk factors, comorbidity, co-medications (e.g., HRT), and drug substances exposed to.

Results. We identified 481 products from 24 drug classes containing either DBP, diethyl phthalate (DEP), cellulose acetate phthalate (CAP), hypromellose phthalate (HPMCP), or polyvinyl acetate phthalate (PVAP). Drugs with phthalate-containing products also included phthalate-free products. Phthalate masses ranged from 3 µg to 1.3 g per pill. We followed 1.12 million women over 9.99 million person-years, during which 27,111 women were diagnosed with invasive breast cancer. Fourteen percent of the cohort (n=161,751) was prescribed a phthalate-containing drug. We observed no breast cancer associations with exposure to CAP, DEP, HPMCP, and PVAP. However, the highest level of cumulative DBP exposure (>10,000 mg; range: 10,024 to 71,340 mg; median=15,390 mg) was associated with an 80% increase in breast cancer risk compared with no exposure (HR_adj=1.8; 95% CI: 1.0, 3.1). The association was strongest for ER+ disease (HR_adj=1.9; 95% CI: 1.1, 3.5) and among premenopausal women (HR_adj=2.2; 95% CI: 0.91, 5.3). There was no evidence of a linear trend in the log-hazard across categories of cumulative DBP exposure. No published evidence links exposure to the drug substances represented by the DBP-containing products (bisacodyl, budesonide, mesalazine, multienzymes, diclofenac, and lithium) with breast cancer risk.

Conclusions. High DBP exposure was associated with increased breast cancer incidence, particularly ER+ disease and among premenopausal women. This association merits further investigation. In the meantime, it may be prudent for women taking DBP-containing medications to substitute a phthalate-free version of the same drug, other considerations being equal.
Title: Comparison of patterns and prognosis among distant metastatic breast cancer patients by age groups: A population-based analysis

Mengting Chen1,2, Hefen Sun1,2, Yang Zhao1,2, Wenyan Fu1,2, Lipeng Yang1,2, Shuiping Gao1,2, Liangdong Li1,2, Honglin Jiang3 and Wei Jin1,2. 1Key Laboratory of Breast Cancer in Shanghai, Fudan University Shanghai Cancer Center, Shanghai, China; 2Shanghai Medical College, Fudan University, Shanghai, China and 3University of Michigan, Michigan.

Body: Background: Distant metastasis has long been the principal cause of mortality among breast cancer patients. Previous studies found that the age of diagnosis probably played important roles in the prognosis of breast cancer, but few researches focused on its roles on metastatic patients and specific organs involvement. The aim of this study is to investigate the disparities of metastatic patterns and prognosis by age groups among stage IV breast cancer patients.

Methods: We collected patients' data from the Surveillance, Epidemiology, and End Results (SEER) database. The patients were selected according to the following criteria: microscopically confirmed primary breast cancer patients, diagnosed between 2010 and 2013, known age at diagnosis, and de novo stage IV (AJCC 7th edition) patients. The clinical characteristics of the selected patients were compared with Pearson's χ² test. The survival curves were compared with log rank test. Cox regression models were used to identify factors which were significantly associated with overall survival (OS) and breast cancer-specific survival (BCSS) among these patients.

Results: We identified 4932 eligible metastatic breast cancer patients, including 850 younger patients (<50 years), 2,540 middle-aged patients (50-69 years) and 1,542 elder patients (>69 years). The median age was 62 years and the median follow-up time was 10 (IQR:2-22) months. The results revealed that younger and middle-aged patients had bigger tumor size, higher rate of lymph node involvement and higher rate of triple negative breast cancer (TNBC) than the elder group (P<0.001). Moreover, elder patients were more likely to have bone and lung metastasis, but less likely to have liver metastasis (P<0.05). Higher proportion of younger (34.9%) and middle-aged (36.2%) patients had multiple metastatic sites than elder patients (28.3%) (P < 0.001). In multivariate analysis, age at diagnosis, race, T stage, molecular subtypes, surgery, radiation therapy, and distant organ metastasis were all significantly associated with prognosis (P < 0.05). Younger patients had better OS (HR: 0.77, 95% CI: 0.68-0.87, P<0.001) and BCSS (HR: 0.81, 95% CI: 0.71-0.92, P=0.002) compared to middle-aged patients, while the elder group had the worst OS (HR: 1.56, 95% CI: 1.43-1.70, P < 0.001) and BCSS (HR:1.52, 95% CI: 1.38-1.68, P < 0.001). Finally, patients with bone metastasis only had superior survival compared to other metastatic patient. Brain metastasis only group and multiple sites metastasis group had the poorest prognosis (P<0.05).

Conclusions: The patients among three age groups presented different metastatic patterns. Age at diagnosis was an independent prognostic factor for metastatic breast cancer patients. Older patients had significantly poorer prognosis. The distinctions among different age groups may lead to more individualized treatment strategies in the future.

Table 1 The 1-year, 2-year survival rate and median survival time (MST) of the metastatic breast cancer patients by age groups

<table>
<thead>
<tr>
<th></th>
<th>OS 1-year</th>
<th>OS 2-year</th>
<th>OS MST (months)</th>
<th>BCSS 1-year</th>
<th>BCSS 2-year</th>
<th>BCSS MST (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;50 years</td>
<td>77.9%</td>
<td>60.9%</td>
<td>32</td>
<td>79.9%</td>
<td>63.7%</td>
<td>37</td>
</tr>
<tr>
<td>50-69 years</td>
<td>71.4%</td>
<td>51.2%</td>
<td>25</td>
<td>75.8%</td>
<td>55.7%</td>
<td>31</td>
</tr>
<tr>
<td>&gt;69 years</td>
<td>56.3%</td>
<td>39.9%</td>
<td>16</td>
<td>63.4%</td>
<td>47.8%</td>
<td>22</td>
</tr>
</tbody>
</table>
Title: Pregnancy associated breast cancer: Evaluating maternal outcomes. A multicentre study

Lisa Prior¹, MinYuen Teo¹, Megan Greally¹, Cian Ward¹, Connor O'Leary¹, Razia Aslam¹, Waseem Darwish¹, Nada Ahmed¹, Geoffrey Watson¹, Deirdre Kelly¹, Lisa Kiely¹, Anees Hassan¹, Jack Gleeson¹, Hannah Featherstone¹, Marvin Lim¹, Hazel Murray¹, David Gallagher¹, Jennifer Westrup¹, Bryan Hennessy¹, Gregory Leonard¹, Liam Grogan¹, Oscar Breathnach¹, Anne Horgan¹, Linda Coate¹, Deirdre O'Mahony¹, Linda Coate¹, Seamus O'Reilly¹, Rajnish Gupta¹, Maccon Keane¹, Karen Duffy¹, Miriam O'Connor¹, John Kennedy¹, John McCaffrey¹, Michaela Higgins¹, Catherine Kelly¹, Desmond Carney¹, Giuseppe Gullo¹, John Crown¹ and Janice Walshe¹. 'Cancer Trials Ireland, Dublin, Ireland.

Body: Background

Pregnancy associated breast cancer (PABC) is defined as breast cancer (BC) diagnosed during the gestational period (GP) or in the first year postpartum (PP). Despite its infrequent occurrence, the incidence of PABC appears to be rising due to the increasing propensity for women to delay childbirth. We have established the first combined prospective and retrospective registry study of PABC in Ireland to examine specific clinicopathological characteristics, treatments and maternal outcomes. We present the retrospective findings to date.

Methods

We performed a retrospective multicentre observational study of patients (pts) with PABC treated in the eight Irish cancer centres from August 2001 to March 2017. Data extracted included information on pt demographics, tumour biology, staging, treatment administered and maternal outcomes. Standard biostatistical methods were used for analysis.

Results

111 PABC patients were identified. Sixty pts (54%) were diagnosed during the GP and 51 (46%) within 1 year PP. Median age at diagnosis was 36 years (yrs). Table 1 illustrates baseline characteristics. Two thirds of pts were node positive and a similar proportion had grade 3 pathology. Seventy pts (63%) were estrogen receptor (ER) positive, 36 (32%) HER2 positive, 25 (22%) triple negative. Twenty-two pts (20%) were metastatic at presentation. Seven pts (6%) had a known BRCA 1/2 mutation. The median OS (overall survival) and DFS (disease free survival) for the entire cohort was 107.4 and 94.2 months respectively (resp). There was no survival difference between those diagnosed during the GP versus PP. 5 yr DFS and OS was 68.6% and 69.2% resp. This compares unfavourably to results reported by the National Cancer Registry of Ireland in a similar age-matched BC population between 2000-2012 where the 5 yr OS was 86.5%. Variables in our study associated with poorer outcomes included younger age, tumour size, node positivity and lack of estrogen expression.

Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>PABC patients (n=11)</th>
<th>Diagnosed in GP (n=60)</th>
<th>Diagnosed 1yr PP (n=51)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td>36</td>
<td>36(25-49)</td>
<td>36(21-44)</td>
<td>0.31</td>
</tr>
<tr>
<td><strong>Stage</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-II</td>
<td>54(60)</td>
<td>55(33)</td>
<td>53(27)</td>
<td>0.85</td>
</tr>
<tr>
<td>III</td>
<td>23(26)</td>
<td>23(14)</td>
<td>23(12)</td>
<td>1</td>
</tr>
<tr>
<td>IV</td>
<td>20(22)</td>
<td>18(11)</td>
<td>22(11)</td>
<td>0.81</td>
</tr>
<tr>
<td>Unknown</td>
<td>3(3)</td>
<td>3(2)</td>
<td>2(1)</td>
<td>1</td>
</tr>
<tr>
<td><strong>Pathology</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>66(74)</td>
<td>70(42)</td>
<td>63(32)</td>
<td>0.43</td>
</tr>
<tr>
<td>Node positive</td>
<td>66(73)</td>
<td>68(41)</td>
<td>63(32)</td>
<td>0.55</td>
</tr>
<tr>
<td>ER+/HER2-</td>
<td>41(45)</td>
<td>38(23)</td>
<td>43(22)</td>
<td>0.69</td>
</tr>
</tbody>
</table>
**ER+/HER2+**  | 23(25) | 28(17) | 16(8) | 0.17  
**ER-/HER2+**  | 14(16) | 17(10) | 12(6) | 0.59  
**Triple negative**  | 22(25) | 17(10) | 29(15) | 0.11  

**Surgery**  
**Breast conservation**  | 23(26) | 25(15) | 21(11) | 0.82  
**Mastectomy**  | 56(63) | 57(34) | 59(30) | 0.84  

**Adjuvant/Neoadjuvant treatment**  
**Chemotherapy**  | 73(81) | 77(46) | 69(35) | 0.39  
**Anthracycline**  | 68(55) | 78(36) | 54(19) | 0.03  
**Taxane**  | 89(72) | 93(43) | 83(29) | 0.16  
**Anti HER2 agent**  | 21(23) | 18(11) | 24(12) | 0.63  
**Endocrine therapy**  | 64(52) | 63(29) | 66(23) | 0.84  
**Radiotherapy**  | 79(64) | 74(34) | 86(30) | 0.85  

**Relapse in Stage I-III**  
**Local relapse**  | 15(13) | 12(6) | 18(7) | 0.55  
**Distant relapse**  | 24(21) | 22(11) | 25(10) | 0.80  

**Conclusions**  
PABC patients may have a poorer outcome. Our study reported higher rates of triple negative and HER2 positive breast cancer which are associated with more aggressive biology. Prospective evaluation of clinicopathological features, pharmacokinetics of treatments selected and maternal and fetal outcomes is imperative in this distinct pt group.
2017 San Antonio Breast Cancer Symposium

Publication Number: P6-08-18

Title: Prognostic multigene testing in breast cancer: Patterns, disparities, and opportunities for advancing standardized patient care

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¹Dartmouth-Hitchcock Medical Center, Lebanon, NH and ²Dartmouth College, Hanover, NH.

Body: Background: The decision to give adjuvant chemotherapy to patients with hormone receptor positive early stage breast cancer is controversial given the overall good prognosis with local therapy (surgery and radiation) plus hormonal therapy alone. In 2004, the 21-gene RT-PCR assay recurrence score (Oncotype) was developed to stratify early stage patients into categories of high, low, and intermediate recurrence rates considering treatment with local and hormonal therapy alone. This was incorporated into the NCCN guidelines in 2008. We sought to compare NCCN guidelines to actual practice patterns.

Methods: By retrospective review, data were examined from eight state registries participating in the National Program of Cancer Registries' Comparative Effectiveness Research program: Alaska, Colorado, Florida, Idaho, Louisiana, North Carolina, New Hampshire, and Rhode Island. These were then compared to NCCN guidelines for prognostic multigene testing.

Results: Of the 28,372 cases examined, 18.6% were classified as carcinoma in situ, 39.6% were stage I, 24.3% were stage II, 9.1% were stage III, 4.9% were stage IV, and 3.6% were unknown stage. The overwhelming majority of cases, 75.5%, were estrogen receptor (ER) or progesterone receptor (PR) positive, while 15.7% were ER and PR negative, and 8.8% were hormone receptor unknown. Approximately 40% of cases were human epidermal growth factor receptor 2 (HER2) positive, and the remaining 60% were HER2 negative or unknown. Approximately 72% of patients were node negative or had unknown nodal involvement, while the remaining 28% had at least micro-metastatic nodal disease. Invasive ductal carcinoma was the most common histology accounting for 71.4% of cases examined. Median age was 62. Data analysis for the use of prognostic multigene testing in relation to NCCN guidelines, race, age, and the above clinical factors is on-going and will be presented at SABCS 2017.

Conclusion: The purpose of this study is to examine the factors associated with the use of prognostic multigene testing according to the NCCN guidelines, including personal and clinical factors. By identifying practice patterns we can then address disparities and opportunities for advancing standardized quality patient care.
2017 San Antonio Breast Cancer Symposium

Publication Number: P6-08-19

Title: Outcomes of patients with invasive breast cancer (IBC) refusing standard cancer treatments: 10-year analysis of the National cancer data base (NCDB)

Amila Orucevic¹, Robert E Heidel¹ and John L Bell¹. ¹The University of Tennessee Medical Center, Knoxville, TN.

Body: Breast cancer treatments (Rx) are becoming more individualized; however, there remain patients (pts) who refuse recommended standard Rx. There is paucity of data defining the characteristics of these pts. Our study objective was to identify socioeconomic characteristics of pts who refuse recommended Rx and analyze their outcome.

Female pts diagnosed with IBC from 2004-2014 were selected from the NCDB. Socioeconomic characteristics and overall survival (OS) of pts who refused standard Rx (surgery, chemotherapy, anti-estrogen therapy, or radiation) were compared with an exact number of randomly matched patients who received recommended Rx. Frequency statistics, Mann-Whitney U test and logistic regression were used in statistical analyses.

There were 1772260 IBC pts; 11164 (0.6%) refused surgery; 108,486 (6.1%) refused chemotherapy; 68,995 (3.9%) refused anti-estrogen; 52,081 (2.9%) refused radiation. Pts who refused recommended Rx were significantly more likely to die, to be older, non-Caucasian race, lower income, and surprisingly higher education than pts who received recommended Rx (p<0.05). Results reveal that older age, non-Caucasian race, and lower income were correlated with refusal of recommended Rx for IBC and consequently significantly worse OS compared to matched pts that received recommended Rx. When putting this into national perspective, this data reveals that almost 10% (~25,000/yr) of newly diagnosed pts with IBC may choose to refuse one or more of recommended standard Rx. Further study is necessary to identify reasons for refusal and ways to minimize these events.

<table>
<thead>
<tr>
<th></th>
<th>Surgery NO</th>
<th>Surgery YES</th>
<th>ChemoRx NO</th>
<th>ChemoRx YES</th>
<th>Anti-estrogen Rx NO†</th>
<th>Anti-estrogen Rx YES†</th>
</tr>
</thead>
<tbody>
<tr>
<td># pts</td>
<td>11164</td>
<td>Random 11164 / 1630429</td>
<td>10846</td>
<td>Random 10846 / 735501</td>
<td>68995</td>
<td>Random 68995 / 1039320</td>
</tr>
<tr>
<td>OS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dead</td>
<td>6004 60.9</td>
<td>1639 16.3</td>
<td>20339 21.7</td>
<td>17159 17.5</td>
<td>12445 20.5</td>
<td>8726 14.2</td>
</tr>
<tr>
<td>Alive</td>
<td>3855 39</td>
<td>8393 83.7</td>
<td>73362 78.3</td>
<td>80710 82.5</td>
<td>48254 79.5</td>
<td>52534 85.8</td>
</tr>
<tr>
<td>Odds ratio for death (95%CI)</td>
<td>7.9 (7.4-8.5)*</td>
<td>1.3 (1.2-1.3)*</td>
<td>1.5 (1.5-1.6)*</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Survival months (mean)</td>
<td>60.8</td>
<td>74</td>
<td>52.8</td>
<td>60</td>
<td>53.3</td>
<td>59.1</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤40</td>
<td>225 2</td>
<td>727 6.5</td>
<td>2602 2.4</td>
<td>11911 11</td>
<td>3210 4.7</td>
<td>3583 5.2</td>
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<tr>
<td>41-69</td>
<td>3507 31.4*</td>
<td>7330 65.7</td>
<td>60764 56*</td>
<td>84835 78.2</td>
<td>36477 52.9**</td>
<td>46575 67.5</td>
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<tr>
<td>70+</td>
<td>7432 66.6*</td>
<td>3107 27.8</td>
<td>45120 41.6*</td>
<td>11740 10.8</td>
<td>29308 42.5**</td>
<td>18837 27.3</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>White</td>
<td>8439 75.6</td>
<td>9414 84.3</td>
<td>92013 84.8</td>
<td>87256 80.4</td>
<td>59542 86.3</td>
<td>59448 86.2</td>
</tr>
<tr>
<td>Black</td>
<td>2162 19.4*</td>
<td>1215 10.9</td>
<td>11190 10.3**</td>
<td>15188 14</td>
<td>6408 9.3 NS</td>
<td>6259 9.1</td>
</tr>
<tr>
<td>Other</td>
<td>408 3.7*</td>
<td>423 3.8</td>
<td>4323 4**</td>
<td>4953 4.6</td>
<td>2369 3.4**</td>
<td>2639 3.8</td>
</tr>
<tr>
<td>Income</td>
<td>155 1.4</td>
<td>112 1</td>
<td>960 0.9</td>
<td>1089 1</td>
<td>676 1</td>
<td>649 0.9</td>
</tr>
<tr>
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<tr>
<td><strong>≤38K</strong></td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td>2239 20.3</td>
<td>1652 14.9</td>
<td>16393 15.2</td>
<td>17146 16</td>
<td>9282 13.6</td>
<td>9645 14.1</td>
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<tr>
<td>38K-47999</td>
<td>2580 23.4**</td>
<td>2359 21.3</td>
<td>24195 22.5*</td>
<td>23071 21.5</td>
<td>14594 21.3*</td>
<td>14473 21.2</td>
</tr>
<tr>
<td>48K-62999</td>
<td>2845 25.8**</td>
<td>2991 27</td>
<td>29646 27.5*</td>
<td>28524 26.6</td>
<td>19335 28.2*</td>
<td>18504 27</td>
</tr>
<tr>
<td>63K+</td>
<td>3378 30.6**</td>
<td>4067 36.7</td>
<td>37489 34.8</td>
<td>38678 36</td>
<td>25282 36.9 NS</td>
<td>25798 37.7</td>
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<tr>
<td><strong>% with NO high school diploma</strong></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>21+</td>
<td>1834 16.6</td>
<td>1578 14.3</td>
<td>14619 13.6</td>
<td>17427 16.2</td>
<td>809111.8</td>
<td>9354 13.7</td>
</tr>
<tr>
<td>13-20.9</td>
<td>2937 26.6</td>
<td>2668 24.1</td>
<td>26426 24.5*</td>
<td>26095 24.3</td>
<td>15697 22.9*</td>
<td>15756 23</td>
</tr>
<tr>
<td>7-12.9</td>
<td>3686 33.4**</td>
<td>3695 33.4</td>
<td>37084 34.4*</td>
<td>35198 32.8</td>
<td>23997 35*</td>
<td>23260 34</td>
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<tr>
<td>&lt;7</td>
<td>2588 23.4**</td>
<td>3131 28.3</td>
<td>29641 27.5*</td>
<td>28738 26.7</td>
<td>20728 30.3*</td>
<td>20080 29.3</td>
</tr>
</tbody>
</table>

\textit{p<.05} *higher or **lower likelihood to refuse Rx (logistic regression, 1st category=referent) NS=Not significant; †Radiation Rx data not shown, similar trends to anti-estrogen Rx
Title: Non-adherers of mammography screening: Delayed surgery, early discontinuation of adjuvant hormone therapy, and worse breast cancer outcomes

Wei He¹, Louise Eriksson Bergman¹², Sven Törnberg³, Fredrik Strand¹⁴, Per Hall¹⁵ and Kamila Czene¹. ¹Karolinska Institutet, Stockholm, Sweden; ²Cancer Center Karolinska, Radiumhemmet, Karolinska Institutet and Karolinska University Hospital, Stockholm, Sweden; ³Stockholm-Gotland Regional Cancer Centre, Stockholm, Sweden; ⁴Karolinska University Hospital, Stockholm, Sweden and ⁵South General Hospital, Stockholm, Sweden.

Body: Purpose: To test the hypothesis that prior non-adherence to mammography screening could predict subsequent non-adherence to breast cancer treatment. Specifically, we hypothesized that as compared with adherers, screening non-adherers may be more likely to have delayed surgery, discontinue their adjuvant hormone therapy, and consequently have worse breast cancer outcomes.

Methods: We conducted a record-linkage study based on data from the Stockholm Mammography Screening Program (1989-2013), Stockholm-Gotland Breast Cancer Register (2001-2017), Swedish Prescribed Drug Register (2005-2017), and Cause of Death Register (2001-2013). Women diagnosed with breast cancer between 2001 and 2008 in Stockholm, Sweden, were prospectively followed for treatment and survival until December 31st, 2013 (N=5106). Screening non-adherers were defined as patients who were invited but did not attend the screening mammography within 2 years before breast cancer diagnosis. Discontinuation of adjuvant hormone therapy was defined as having any interval between two consecutive dispenses exceeding 180 days during follow-up. Disease-free survival was defined as time to local recurrence, distant metastasis, contralateral breast cancer, or death from breast cancer, whichever came first.

Results: The proportion of delayed surgery (≥6 weeks) for screening adherers (restricted to interval cancers only) versus non-adherers were 15.5% versus 20.6%, respectively (P<0.01). As compared with adherers, screening non-adherers were more likely to discontinue their adjuvant hormone therapy, with an adjusted hazard ratio of 1.37 (95% CI, 1.17 to 1.61). Furthermore, Cox regression analysis showed that screening non-adherence was associated with poorer disease-free survival even after adjusting for tumor characteristics and other covariates, with an adjusted hazard ratio of 1.19 (95% CI, 1.01 to 1.41) for screening non-adherers versus adherers.

<table>
<thead>
<tr>
<th></th>
<th>Screening Adherers</th>
<th>Screening Non-adherers</th>
<th>Univariable-analysis</th>
<th>Multivariable analysis#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delayed surgery&gt;6 weeks*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>897(84.5)</td>
<td>624(79.4)</td>
<td>1.00 (Reference)</td>
<td>1.00(Reference)</td>
</tr>
<tr>
<td>Yes†</td>
<td>165(15.5)</td>
<td>162(20.6)</td>
<td>1.40(1.10-1.78)</td>
<td>1.34(1.05-1.72)</td>
</tr>
<tr>
<td>Discontinuation of adjuvant hormone therapy**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>822(50.3)</td>
<td>145(42.7)</td>
<td>1.00(Reference)</td>
<td>1.00(Reference)</td>
</tr>
<tr>
<td>Yes††</td>
<td>812(49.7)</td>
<td>195(57.3)</td>
<td>1.33(1.14-1.56)</td>
<td>1.37(1.17-1.61)</td>
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<tr>
<td>Disease free survival events</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>3574(85.8)</td>
<td>752(79.8)</td>
<td>1.00(Reference)</td>
<td>1.00(Reference)</td>
</tr>
<tr>
<td>Yes††</td>
<td>590(14.2)</td>
<td>190(20.2)</td>
<td>1.46(1.24-1.72)</td>
<td>1.19(1.01-1.41)</td>
</tr>
</tbody>
</table>

* Restricted to patients diagnosed with clinically detected cancers who were primary operated without neoadjuvant therapy; ** Restricted to patients diagnosed after July 1st, 2005, who initiated adjuvant hormone therapy.† Odds ratios (95% CI); † † Hazard ratios (95% CI).# Adjusting for age, tumor size, lymph node involvement, estrogen receptor status, progesterone receptor status, HER2 status, and type of surgery
Conclusions: Compared to adherers, screening non-adherers are more likely to have delayed surgery, discontinue their adjuvant hormone therapy, and subsequently have worse breast cancer outcomes even after adjusting for baseline tumor characteristics.
Body: Background and Aim:
The five-year survival for women with stage I-II breast cancer is 93-100%. Despite standard of care treatment, a small subset of these women suffer early breast cancer-specific mortality and die within 12 months of diagnosis. This subset of women has not been previously described. The aim of this study is to characterize the incidence, demographics, and clinical characteristics of women with early stage breast cancer who suffer early breast cancer-specific mortality.

Methods:
Retrospective population study of the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) registry of women diagnosed with stage I, IIA, or IIB breast cancer between 2004 and 2010. Data were filtered to histology codes 8500-8543 and 8575. Patient demographics (age, race, ethnicity) and clinical characteristics (stage, T stage, N stage, grade, ER status, PR status) of women in the early mortality subset were compared with those of women who survived > 12 months via the Chi-square test and the student t-test.

Results:
259,380 women formed the basis of our analysis. 4,572 women (0.018%) died within 12 months of diagnosis. Compared with those who survived > 12 months, women who suffered early breast cancer-specific mortality were on average older (mean age 65.7 years versus 60.3 years, p<0.00001) and more likely to be Hispanic (14.3% versus 8.9%, p<0.00001) or black (11.0% versus 9.1%, p<0.00001). Clinical characteristics associated with early mortality included higher stage (stage IIA 34.2% versus 29.4%, stage IIB 21.8% versus 12.9%, p<0.00001), higher T stage (T2 40.5% versus 28.1%, T3 3.1% versus 1.6%, p<0.00001), higher N stage (N1 29.7% versus 23.2%, N2 0.8% versus 0.4%, N3 0.4% versus 0.1%, p<0.00001), higher grade (moderate 39.3% versus 42.5%, high 40.0% versus 31.5%, p<0.00001), higher rates of ER negativity (27.2% versus 19.0%, p<0.00001), and higher rates of PR negativity (38.5% versus 30.2%, p<0.00001).

Conclusions:
Breast cancer-specific mortality within 12 months of diagnosis of stage I-II breast cancer is a rare phenomenon which has not been previously characterized. There are several demographic and clinical features associated with early mortality, however further research is needed to identify specific prognostic factors that will allow identification of women at risk for early mortality at the time of diagnosis.
Body: Background

In 2013 the ASCO/CAP Guidelines for HER2 testing were updated and revised to improve the accuracy of Her2 testing and its utility as a predictive marker in breast cancer. In this prospective cross-sectional study, we assessed the proportion of Her2 positive and equivocal cases in a real-world setting in Germany. Patients' characteristics and tumor biological factors were evaluated. Furthermore, we analyzed the effect of the updated recommendations on the clinical management.

Methods

This IRB approved prospective analysis included patients with primary breast cancer. Patients with primary stage IV and recurrent breast cancer were excluded. Data was collected from 2012-2017 in six certified breast centers using a personal questionnaire and data from the patients' medical records.

Results

2705 primary breast cancer cases were analyzed. The groups consisted of 320 (11.8%) TNBC, 1956 (72.31%) luminal breast cancer patients and 381 (14.08%) Her2 positive cancer patients. 18 (1%) patients were classified as equivocal. 30 patients did not meet the criteria of any of these groups. No significant difference was seen in positive lymph node status between all groups. Tumor size at diagnosis was larger for TNBC compared to the other groups. 81.9% of all TNBC, 65.1% of the Her2 type, 44.2% of the luminal type and 44.44% (8/18) of the equivocal cases were grade 3 tumors. 3 cases with unresolved Her2 status were diagnosed in 2013 and 6 cases in 2016. 17/18 equivocal cases were hormone receptor positive, 1/18 was hormone receptor negative. In 12/18 cases adjuvant or neoadjuvant chemotherapy was recommended. Trastuzumab was recommended in 9/18 of the cases. 2/18 patients were BRCA mutation carriers. 2/18 received an oncologic treatment for another cancer prior to their breast cancer diagnosis. 1/18 patient received primary endocrine therapy with an aromatase inhibitor. 6/18 patients had a positive family history for breast cancer.

Conclusions

Our study indicates that the 2013 ASCO/CAP update does not affect the overall Her2 positivity rate in breast cancer. Although the proportion of tests and retests increased, only few unresolved cases remained. Despite the fact that the benefit of anti-Her2 directed therapy in equivocal cases is unproven, oncologists were more likely to recommend Trastuzumab, if chemotherapy was indicated for high risk breast cancer. There are numerous factors that could explain equivocal findings. Interestingly, a significant number of our patients presented endogenous or exogenous risk factors for chromosomal aberrations that might be responsible for unresolved Her2 cases.
Title: Delayed surgery and its impact on survivorship in Southeast-Asian women with operable breast cancer – a population based study

Peh Joo Ho¹, Jenny Liu¹, Hui Miao¹ and Mikael Hartman¹.
¹Saw Swee Hock School of Public Health, National University of Singapore, Singapore, Singapore.

Body: Introduction
Ensuring short interval (<3 month) between diagnosis and commencement of surgery for breast cancer may become challenging with the increasing incidence of breast cancer in Southeast-Asia. We aim to study the impact of delayed surgery on breast cancer specific death in Southeast-Asian women diagnosed with breast cancer.

Methods
Population-based data of women diagnosed with breast cancer between 2005 and 2011 was obtained from the National Registry of Disease Office, Singapore. 1816 women with in situ breast cancer and 7896 women with a biopsy or surgical diagnosis of stage I -III breast cancer, who had surgery within 6 months of diagnosis, were included in this study. Surgery which occurred 3 months post-diagnosis was considered as a delay in receiving surgery. Time since diagnosis was calculated as the interval between diagnosis and date of death or end of study (24 May 2016) whichever is earlier. We are interested in breast cancer specific death (ICD9: 174, ICD10: C50). Cox proportional hazard model was fitted to study the association between survival and delayed surgery (surgery ≤3 months versus surgery in 3-6 months post diagnosis), adjusted for age at diagnosis, ethnicity, tumor grade, and year of diagnosis.

Results
Median age at diagnosis was 51 (IQR: 45 – 59) in women diagnosed with in situ breast cancer and 53 (IQR: 46 – 61) in women diagnosed with stage I – III breast cancer. The median survival time was 8 years (IQR: 6 – 9) and 7 years (IQR: 5 – 9) in women diagnosed with in situ and stage I – III breast cancer, respectively. Among women with invasive cancer, 3144 (40%) were diagnosed at stage I, 3420 (43%) at stage II, and 1332 (17%) at stage III. In women with in situ breast cancer, 1043 (57.4%) received treatment within 3 months of diagnosis, and 29 (2%) died of breast cancer. The risk of breast cancer specific death in women who received treatment after 3 months or did not receive treatment was 2.3 (95%CI: 1.1 – 4.8) times that of those who received treatment within 3 months, however the absolute difference in risk at 5 and 10 year post diagnosis was 1.1 and 1.6%, respectively. In women diagnosed with stage I – III breast cancer, 7759 (98%) received surgery within 3 months of diagnosis, while 137 (2%) had delayed surgery. 694 (9%) of women who received surgery within 3 months and 22 (16%) who had delayed surgery died of breast cancer. The risk of breast cancer specific death of women with delayed surgery was 1.7 (95%CI: 1.1 – 2.6) times that of women who received surgery within 3 months post-diagnosis, adjusted for age at diagnosis, ethnicity, tumor grade, and year of diagnosis. However, the absolute difference in risk for breast cancer death at 5 and 10 year, between delayed surgery and having surgery within 3 months, was 0.7 and 1.7%, respectively.

Conclusion
Delayed surgery is rare (2%) in women diagnosed with stage I – III operable breast cancer. The risk of breast cancer specific death among women with delayed surgery is 70% higher than women who received surgery within 3 months of diagnosis, however the absolute difference in risk of breast cancer death is small, <2%. Achieving surgery within 3 months post diagnosis may have marginal improvement in survival.
Title: Reproductive status and clinical pathological characteristics of young women diagnosed with breast cancer in Latin America: LACOG 0414 study

Gustavo Werutsky¹, Cynthia Villarreal-Garza²,³,⁴, Zaida D Morante Cruz⁶,⁷, Marcio Debiasi¹, Facundo Zaffaroni¹, Alan Fonseca², Andrea Castro-Sánchez²,⁵, Alejandra Platas²,³, Henry Gómez Moreno⁶,⁷, Denisse Bretel⁶,⁷, Rosa M Ortiz⁸, Tomás Reinert⁹, Vanessa Dybal¹⁰, Pedro Liedke¹¹ and Carlos Barrios¹. ¹Latin American Cooperative Oncology Group (LACOG), Porto Alegre, Brazil; ²Joven & Fuerte: Programa para la Atención e Investigación de Mujeres Jóvenes con Cáncer de Mama, Mexico City, Mexico; ³Instituto Nacional de Cancerología, Mexico City, Mexico; ⁴Centro de Cancer de Mama, Tecnológico de Monterrey, Monterrey, Mexico; ⁵Catedras CONACYT, Instituto Nacional de Cancerología, Mexico City, Mexico; ⁶Instituto Nacional de Enfermedades Neoplasicas (INEN), Lima, Peru; ⁷Grupo de Estudios Clinicos Oncologicos Peruano (GECOPERU), Lima, Peru; ⁸Instituto Nacional de Oncologia y Radiobiologia, Havana, Cuba; ⁹Centro de Pesquisa e Educação da Serra Gaúcha (CEPESG), Caxias do Sul, Brazil; ¹⁰Clínica AMO, Salvador, Brazil and ¹¹Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil.

Body: BACKGROUND
Approximately 7% of women diagnosed with breast cancer (BC) are under 40 years old. Chemotherapy may adversely affect ovarian function therefore fertility is an issue to be addressed for these patients. Patients with cancer in Latin America have limited access to fertility preservation. However age at first birth in women from Latin America is lower than in developed country and the needs of fertility preservation may be different from developed countries. The aim of the present study was to describe the reproductive status and clinical pathological features of young women diagnosed with BC in Latin America.

METHODS
LACOG 0414 is a prospective registry in Latin America which included young patients with < 40 years old with diagnosis of BC and indication of (neo) adjuvant chemotherapy. Demographic data, reproductive status and clinical pathological information were retrieved from patients’ medical charts. This study was approved by local ethics committees and regulatory authorities.

RESULTS
A total of 343 patients (pts) from 19 different sites distributed in 4 Latin American countries were included: Brasil (N=132 pts), Mexico (N=112 pts), Peru (N=83 pts) and Cuba (N=16 pts). The mean age at BC diagnosis was 34.02 (±4.17) years (14% had < 30 years, 41% had 30-35 years and 44% had 36-40 years). From all included patients, 245 (71.42%) already had children at the time of BC diagnosis. From those the median number of children per women was 2 (range 1–6). The probability of having children at the time of diagnosis was significantly higher with increased age, being as high as 80% in those aged between 36 and 40 years old. There was no difference in terms of having a child at BC diagnosis within the 4 countries, by stage at diagnosis or per breast cancer subtypes.

In terms of educational level, approximately 8% of patients were illiterate and only 40% had university degree. Stages at BC diagnosis were the following: 12% stage I, 49% stage II, 35% stage III and 3% stage IV. The distribution of BC subtypes was: luminal 48%, HER2-positive 29% and triple negative 23%.

CONCLUSION
To our knowledge, this is the first study describing reproductive status of young patients diagnosed with BC in Latin America. Despite the lack of access to fertility preservation programs in the region, we found that a high number of patients had children at diagnosis of BC. Considering the high prevalence of advanced disease and aggressive subtypes the socioeconomic impact of young BC patients in Latin America needs to be addressed.
Title: Outcome of breast cancer patients treated in the private health care in Brazil

Alexandre Boukai1,2, Aline C Gonçalves1,2, Perla M Andrade1, Natalia Carvalho1, Thamires Almeida1, Flávio Lemos1, Monica Padoan1, Nelson S Teich1,2 and Luiz H Araujo1,2. 1Instituto COI de Educação e Pesquisa and 2Americas Centro de Oncologia Integrado.

Body: Background
Developing countries like Brazil often present a dichotomous health care system, where patients may be treated in either public or private institutions that differ substantially in terms of level of access to diagnostic and therapeutics procedures. Herein, we present the first report of a comprehensive study to assess real-world data in breast cancer patients treated in the private health care in Brazil.

Methods
This is a prospective study of breast cancer patients treated in a private health care institution, comprising six unities in Rio de Janeiro and surroundings. Eligible patients were at least 18 years old and had a histology-proven diagnosis of breast cancer between July 2012 and November 2016. For this analysis, only female patients with an invasive component were included. Patients with prior malignancies and those who initiated therapy in other institutions were excluded. Patients or relatives were contacted by telephone to ensure that all information was annotated. Data quality was certified by regular monitoring. This study was approved by the local Research Ethics Committee.

Results
One thousand three hundred and ninety patients were enrolled. One hundred sixty were excluded in this analysis, 11 due to male gender and 151 with exclusively in situ carcinoma. The report comprises 1230 female patients, predominantly diagnosed in early (79.0% stages I-II) or locally advanced (16.1% stage III) stages. One thousand thirty-three (84.0%) patients had hormone receptor (HR)-positive tumors, and 185 (15.0%) were HER2 positive. One hundred twenty-six (10.2%) cases were triple negative. The primary tumor was resected in 89.0% of times, most often through breast-conserving surgery (55.1%). Axillary lymph nodes were assessed in 83.4% of cases, and 32.0% required complete axillary dissection. Chemotherapy was used in 61.3% of cases, and radiation therapy in 59.6%. Patients with locally advanced disease received more aggressive therapy than patients with early stage (higher rates of mastectomy, axillary dissection and chemotherapy use). After a median follow up of 22.5 months (95% CI, 21.09-23.90), 54 (4.4%) deaths were reported. The estimated 2-year overall survival (OS) was 95.3%. 2-year OS was significantly longer among patients with stages I-II (97.9% and 97.5%, respectively) than in stages III and IV (89.4% and 69.5%, respectively) (p<0.01). HER2/HR status (p<0.01) and tumor grade (p=0.05) were also correlated to OS in the overall cohort, however triple-negative cases were only prognostic in stage III. Age (p=0.10), menopausal status (p=0.74), and histological subtype (p=0.55) were not correlated to OS.

Conclusion
To our knowledge, this is the most comprehensive and best-annotated study in breast cancer patients treated in the private health care in Brazil. More oncological interventions were used in advanced stages, reflecting international recommendations, but also a need to pursue early diagnoses, where outcome is optimal despite less aggressive therapy. Outcomes are favorably similar to the current literature from developed countries in all stages. The data provided helps comprehending the current scenario of breast cancer presentation and treatment in Brazil, and may serve as a foundation to guide resource allocation in the years to come.
Outcomes and prognostic factors in 2000 patients with TNBC: Long-term results covering 10 years


Body: Background: Triple-negative breast cancer (TNBC) is characterized by being a heterogeneous disease with different risk factors and poor survival rates. TNBC have a higher prevalence in the Peruvian population (21.3%) than in Caucasian groups. Our study aims to determine the outcome and identify prognostic factors in Peruvian women with TNBC.

Methods: We retrospectively analyzed TNBC patients treated at the “Instituto Nacional de Enfermedades Neoplasicas” between 2000 and 2014. Survival rates and differences were calculated by the Kaplan-Meier method and Log-rank test, respectively. With the Cox regression, in univariate and multivariate analysis, we identified prognostic factors in for our TNBC population.

Results: In total, 2007 patients were diagnosed. The mean age was 50 years (range: 19-95 years); 44.8% were premenopausal and 26.2% had obesity at diagnosis. A family history of breast and/or ovarian cancer was present in 266 (28.9%) patients. Regarding the clinical-pathological features, 1860 (93.5%) had ductal invasive carcinoma and 1024 (51.6%) patients were in Stage (S) III. Local relapse and distant metastasis affected to 34.5% and 51.4% of our patients, respectively. Lung (14.5%) and bone (9.7%) were the most frequent sites of metastasis. The median follow-up was 9 years. The 5 and 10 yrs DFS/OS rates are shown in table 1. In the multivariate analysis, adjuvant chemotherapy (ACT) (HR: 0.60, 95%CI: 0.44-0.82, p=0.001) and radiotherapy (RT) (HR:0.72, 95%CI:0.55-0.93, p=0.014) were associated with a reduced risk of recurrence, while nodal involvement were associated to a high recurrence risk. Factors associated to a reduction in the risk of death were ACT (HR:0.73, 95%CI:0.61-0.88, p=0.001), RT (HR: 0.70, 95%CI: 0.60-0.92, p<0.001), and Neoadjuvant CT (HR: 0.61, 95%CI: 0.51-0.73, p<0.001); in contrast, a NLR≥3 (HR:1.60,95%CI: 1.36-1.87, p<0.001), N stage were associated with a higher risk of death.

Conclusions: Sociodemographic features of Peruvian patients with TNBC resemble other populations; however, our population is diagnosed at more advanced clinical stages, hence DFS and OS were lower than international reports while prognostic factors were similar to previous studies.

Table 1. 5- and 10-years DFS/OS rates

<table>
<thead>
<tr>
<th>Clinical Stage</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>Total</th>
<th>p (value)</th>
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</thead>
<tbody>
<tr>
<td>DFS (%) 5-years</td>
<td>83</td>
<td>75</td>
<td>40</td>
<td>-</td>
<td>63</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>10-years</td>
<td>69</td>
<td>64</td>
<td>29</td>
<td>-</td>
<td>52</td>
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</tr>
<tr>
<td>OS (%) 5-years</td>
<td>93</td>
<td>82</td>
<td>39</td>
<td>6</td>
<td>55</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>10-years</td>
<td>83</td>
<td>75</td>
<td>32</td>
<td>5</td>
<td>50</td>
<td></td>
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</tbody>
</table>
Title: Development of a clinical registry for breast cancer in Norway

Olaf Johan Hartmann-Johnsen1, Ellen Schlichting2, Rolf Kåresen3 and Jan Franz Nygård2. 1Kalnes Hospital, Sarpsborg, Norway; 2Cancer Registry of Norway, Oslo, Norway and 3Oslo University Hospital, Oslo, Norway.

Body: Background: Increased focus on Quality Indicators and Clinical quality registries shows higher compliance to recommended therapy and increased survival. Furthermore, a clinical registry offers an ideal infrastructure for enrolling and following patients in randomized clinical trials. To achieve EUSOMA certification of a Breast Cancer Unit, list of 14 Quality Indicators selected for certification purposes are mandatory. The Norwegian Breast Cancer Group (NBCG) wants a tool for evaluating compliance and results of recommended treatment in breast cancer care on a regular basis utilising the Norwegian Clinical Registry for Breast Cancer run by the Cancer Registry of Norway. The main objective of this study is to describe the development and practise of the Norwegian Clinical Registry for Breast Cancer and to facilitate EUSOMA approval for breast cancer unites on a national level. Report can be given for every institution treating breast cancer in Norway.

Methods: To provide researchers with high quality cancer data as well as for the purpose of national cancer statistics, the Cancer Registry of Norway (CRN) employs a cancer registry system to 1) longitudinal collection of data from all patients from all medical entities that diagnosis and/or treat cancer patients (e.g., pathology laboratories and clinic hospitals) in Norway; 2) validate the correctness of collected data, and 3) assemble the validated cancer data as cancer cases.

Results: All hospitals currently operating breast cancer patients provides data for the EUSOMA criteria. Data from pathology and surgery are of high quality, However, data from oncologic departments are lacking, but improving. We are however confident that these data will be reported regularly within the next years. We can now provide 8 of 14 mandatory Quality Indicators.

<table>
<thead>
<tr>
<th>Quality Indicator</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coverage of patients reported to the breast cancer registry</td>
<td>99.9%</td>
</tr>
<tr>
<td>Proportion with reports on diagnosis (TNM stage)</td>
<td>90%</td>
</tr>
<tr>
<td>Proportion with reports on surgical treatment</td>
<td>89%</td>
</tr>
<tr>
<td>Proportion with reports regarding radiology treatment</td>
<td>37%</td>
</tr>
<tr>
<td>5-years relative survival. Estimated with patients who lived in 2014-2016</td>
<td>90.4%</td>
</tr>
<tr>
<td>Proportion with preoperativ diagnosis confirmed with biopsy or cytology</td>
<td>98% Eusoma</td>
</tr>
<tr>
<td>Proportion with DCIS where grading is reported</td>
<td>98.9% Eusoma</td>
</tr>
<tr>
<td>Proportion of patients with invasive cancer and axillary clearance with at last 10 lymph nodes examined</td>
<td>77% Eusoma</td>
</tr>
<tr>
<td>Proportion of patients with invasive cancer not grater than 3 cm who underwent BCT (Neoadjuvant excluded)</td>
<td>81.5% Eusoma</td>
</tr>
<tr>
<td>Proportion of patients with non invasive breast cancer not grater than 2 cm who underwent BCT</td>
<td>90.6% Eusoma</td>
</tr>
<tr>
<td>Proportion of patients with DCIS who do not undergo axillary clearance</td>
<td>100% Eusoma</td>
</tr>
<tr>
<td>Proportion of patients (DCIS) who received just one operation</td>
<td>95.5% Eusoma</td>
</tr>
<tr>
<td>Proportion of invasive breast cancer patients with pN0 who do not undergo axillary clearance</td>
<td>98.2% Eusoma</td>
</tr>
</tbody>
</table>

Conclusion: The NBCR promises to deliver all 14 EUSOMA criteria to all hospitals in Norway on a regular annual basis, thus facilitating EUSOMA approval for breast cancer unites.
**Title:** Risk-reducing oophorectomy and breast cancer risk across the spectrum of familial risk using a prospective family study cohort (ProF-SC)

Mary B Terry¹,², Kelly A Phillips³,⁴,⁵, Mary B Daly⁶, Irene L Andrulis⁹, Yuyan Liao¹, Xinran Ma¹, Nur Zeinomar¹, Robert J MacInnis⁴, Gillian S Dite⁴, Esther M John⁷,⁸, Saundra S Buys¹⁰ and John L Hopper⁴. ¹Columbia University Mailman School of Public Health, New York, NY; ²Herbert Irving Comprehensive Cancer Center, Columbia University Medical Center, New York, NY; ³The University of Melbourne, Melbourne, Australia; ⁴School of Population and Global Health, The University of Melbourne, Melbourne, Australia; ⁵Peter MacCallum Cancer Centre, Melbourne, Australia; ⁶Fox Chase Cancer Center, Philadelphia, PA; ⁷Cancer Prevention Institute of California, Fremont, CA; ⁸Stanford University School of Medicine, Stanford, CA; ⁹Lunenfeld-Tanenbaum Research Institute, University of Toronto, Toronto, ON, Canada and ¹⁰Huntsman Cancer Institute, University of Utah School of Medicine, Salt Lake City, UT.

**Body:**

**Background:** Whether risk-reducing salpingo oophorectomy (RRSO) reduces breast cancer risk in addition to reducing ovarian cancer risk is controversial with some arguing that the previous evidence of a reduction in breast cancer risk from RRSO was due to bias. Evidence from independent prospective cohorts of high-risk women is needed to resolve this controversy.

**Methods:** Using a prospective family study cohort of 17,810 women unaffected with breast cancer at baseline, we examined the association between RRSO and breast cancer risk using Cox Proportional Hazards models. We compared results estimating RRSO as a non-time-dependent variable to results treating RRSO as a time-dependent variable, because failing to account for the time-varying nature of a covariate person-time prior to RRSO, should it exist, will incorrectly attribute the cancer-free person-time to RRSO. We separately examined the association with RRSO in BRCA1 and BRCA2 mutation carriers and non-carriers, and further performed gene-stratified analyses in women with BRCA1 and BRCA2 only. We also assessed multiplicative interactions with underlying familial risk profile (FRP), defined as total lifetime risk estimated from the Breast Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm (BOADICEA) model.

**Results:** During a median 10.7 years of follow-up (maximum 23.7 years), we observed 1,040 incident cases of breast cancer with an average age at diagnosis of 55.8 years and average age at enrollment into the cohort of 46.8 years. A total of 2434 (14%) women reported at baseline having a RRSO. We observed decreased risk of breast cancer associated with RRSO for both BRCA1 (N= 650) and BRCA2 (N=557) mutation carriers when RRSO was treated as a fixed covariate (HR= 0.60, 95% CI=0.40-0.92 and HR= 0.40, 95%CI = 0.23-0.69, respectively). In contrast, when we treated RRSO as a time-varying covariate, for both BRCA1 and BRCA2 carriers, we no longer observed a decreased risk for BRCA1 and BRCA2 carriers (HR= 1.67, 95% CI=1.05-2.67 and HR= 0.97, 95%CI = 0.53-1.80, respectively). There was no association between RRSO and breast cancer risk for non-carriers (N=16,603), whether we treated RRSO as a fixed or time varying covariate (HR= 0.88, 95% CI=0.72-1.08 and HR= 1.06, 95%CI = 0.85-1.30, respectively).

**Conclusions:** Our findings provide an independent replication that the reduced risk of breast cancer previously observed in BRCA1 and BRCA2 mutation carrier women may be from bias in counting person-time. Clinical management of high-risk women should counsel based on the reduced risk of ovarian cancer from RRSO, but not breast cancer.
Title: Associations between hereditary cancer panel predisposition genes and breast cancer histological subtypes

Jenna Lilyquist¹, Holly Laduca², Chunling Hu¹, Jie Na¹, Eric C Polley¹, Steven N Hart¹, Tina Pesaran², Brigette Tippin-Davis², David E Goldgar³, Jill S Dolinsky² and Fergus J Couch¹. ¹Mayo Clinic, Rochester, MN; ²Ambry Genetics, Aliso Viejo, CA and ³University of Utah, Salt Lake, UT.

Body: Background: Clinical panel testing has become routine practice for patients that are diagnosed with breast cancer at a young age and/or have a personal or family history of cancer. Associations with known breast cancer genes and breast cancer subtypes have been previously identified, such as \textit{BRCA1} associations with estrogen receptor negative (ER-) and triple negative (ER-/PR-/HER2-) breast cancers. However, the cancer predisposition genes associated with each of the four clinical subtypes of breast cancer have not been fully defined. We evaluated 24,901 Caucasian female breast cancer cases receiving clinical panel testing for 23 cancer predisposition genes and assessed associations between mutations in each gene and breast cancer subtypes.

Methods: Germline hereditary cancer multigene panel testing results for cancer predisposition genes were obtained for 24,901 Caucasian female breast cancer cases evaluated by a clinical testing laboratory. Information on tumor histology, personal and family history of cancer, age at diagnosis, and previous genetic testing was provided by clinical care providers of patients receiving clinical cancer genetic testing. Breast cancer cases were classified into clinical breast cancer subtypes based on estrogen/progesterone hormone receptor status (HR) and HER2 status: Luminal A (HR+/HER2-), Luminal B (HR+/HER2+), HER2 subtype (HR-/HER2+), and Triple Negative (HR-/HER2-). The frequency of pathogenic or likely pathogenic mutations observed in each subtype was compared against the Exome Aggregation Consortium (ExAC) non-TCGA non-Finnish European population to estimate risks.

Results: \textit{ATM} was associated with moderate risks (odds ratio (OR)>2.0) of Luminal A, Luminal B, and HER2 subtypes of breast cancer, but was not associated with the Triple Negative subtype. \textit{PALB2} was associated with moderate risk for Luminal B subtype, but high risk (OR>5.0) for Luminal A, HER2, and triple negative subtypes. \textit{TP53} was associated with high risks for Luminal B and HER2 tumors. \textit{NBN}, \textit{MRE11A}, and \textit{RAD50} were not associated with any subtype of breast cancer.

Conclusions: Identifying associations between inherited mutations (odds ratio (OR)>2.0) and breast cancer subtypes can inform clinical risk management, treatment options, and therapeutic development efforts.
Title: Gene expression patterns in younger versus older HR+ breast cancer patients: An age-related analysis of HoxB13/IL17BR (H/I), proliferation status, and quantitative hormone receptor expression

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Body: Background: HR+ breast cancer in younger vs older patients may have distinct biological features. The Breast Cancer Index (BCI) is a gene expression-based assay that includes two component biomarkers: the HoxB13/IL17BR (H/I ratio), an endocrine response biomarker; and the molecular grade index (MGI), a set of proliferation-related genes. The objective of this study was to assess age-related associations with endocrine sensitivity (H/I), proliferative status (MGI), and quantitative expression of ER and PR.

Methods: Data were extracted from the BCI Clinical Database for Correlative Studies, an IRB-approved de-identified database containing clinicopathologic and molecular variables from clinical cases submitted for BCI testing. Molecular results from H/I, MGI, and quantitative (qPCR–based) ER and PR were analyzed across age groups. Chi-squared tests and ANOVA were used to compare the results between age groups (<40y, 40-49y, 50-59y, 60-69y, and ≥70y).

Results: Analyses included 19,126 patients (median age at diagnosis 58.6y; 4.5% <40y, 20.6% 40-49y, 28.9% 50-59y, 32.5% 60-69y, and 13.6% ≥70y). Proliferation status (MGI) was significantly higher in patients <40y and 40-49y compared to older groups (P<.0001). H/I analysis indicated a similar distribution of high versus low endocrine responsiveness across all groups (P=.94), except the 40-49y group, in which fewer patients had high H/I (44.2% in <40y, 39.4% in 40-49y, 43.7% in 50-59y, 43.7% in 60-69y, and 43.1% in ≥70y; P=.0001). Median qER increased with age (P<0.0001), while qPR was similar across all age groups except for the 40-49y group (P=.57), in which expression was higher (P<.0001).

Conclusion: Results from >19,000 patients with early-stage HR+ breast cancer and BCI testing showed a broad distribution in all variables. Tumors from the youngest patients (<40y) had the highest expression of proliferative genes and the lowest quantitative ER expression. However, endocrine response, according to the H/I biomarker, does not appear to be strongly linked to age.
Title: Benign breast disease and breast cancer risk across the spectrum of familial risk using a prospective family study cohort (ProF-SC)

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Body: Background: Benign breast disease (BBD) is one of the strongest risk factors for breast cancer but it is unclear whether the strength of the association with BBD and breast cancers varies by breast cancer family history. Few studies of BBD enrich specifically for putative genetic factors by over-sampling based on family history let alone evaluate potential interactions with measures of underlying familial risk. The aim of this study was to evaluate how risk associated with BBD is modified by underlying familial risk so as to guide clinical management and risk assessment of women with BBD.

Methods: Using a prospective family study cohort of 17,154 women unaffected with breast cancer at baseline and followed by questionnaire at regular intervals, we examined the association between BBD and breast cancer risk using Cox Proportional Hazards models. We classified women as having BBD if they reported at baseline having been told by a doctor that they had BBD, such as a non-cancerous cyst or breast lump. We did not have information on histologic sub-type. We confirmed self-reported diagnosis of BBD with pathology reports in a subset of the New York cohort and found high agreement between self-reported and pathologically confirmed BBD (93.5%). We assessed multiplicative and additive interactions with underlying familial risk profile (FRP) defined as either fixed-time horizon of 1-year, or total lifetime risk, estimated from the Breast Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm (BOADICEA) model.

Results: During 176,756 person-years of follow-up (mean 10.2, maximum 23.7 years), we observed 968 incident breast cancers cases with an average age at diagnosis of 55.8 years and average age at enrollment into the cohort of 46.8 years. At baseline, 4,704 (27%) women reported having a previous diagnosis of BBD. Compared to women with no history of BBD, breast cancer risk was increased in women of all ages (HR: 1.37, 95% CI: 1.19, 1.56), and in women up to age 45 years (using attained age models) (HR: 1.40, 95% CI: 1.01, 1.93). In terms of recency of BBD, we found that the increased risk associated with BBD remained 21 years or more after the initial BBD diagnosis (HR: 1.37, 95% CI: 1.11, 1.68). We found no evidence for multiplicative interactions with FRP, which implies that the increase in absolute risk associated with BBD depends on a woman's FRP (Table 1).

Conclusions: Women with a history of BBD have an increased risk of breast cancer that multiplies their underlying familial risk (FRP). These results could prove to be valuable for risk counseling and clinical management.

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Table 1: Cumulative Incidence of Breast Cancer to age 45, 55, and 65 by BBD and underlying FRP as measured by 10-year BOADICEA score.

<table>
<thead>
<tr>
<th>Age</th>
<th>No BBD, &lt;3.4%</th>
<th>BBD, &lt;3.4%</th>
<th>No BBD, ≥3.4%</th>
<th>BBD, ≥3.4%</th>
</tr>
</thead>
<tbody>
<tr>
<td>45</td>
<td>4.6 (3.8, 5.6)</td>
<td>6.1 (4.7, 8.0)</td>
<td>12.1 (10.2, 14.5)</td>
<td>16.1 (13.1, 19.7)</td>
</tr>
<tr>
<td>55</td>
<td>7.4 (6.3, 8.7)</td>
<td>9.8 (7.5, 12.8)</td>
<td>19.1 (16.6, 22.0)</td>
<td>25.0 (21.7, 28.9)</td>
</tr>
<tr>
<td>65</td>
<td>9.7 (8.2, 11.5)</td>
<td>12.8 (9.9, 16.5)</td>
<td>24.5 (21.8, 27.6)</td>
<td>31.8 (28.3, 35.7)</td>
</tr>
</tbody>
</table>
Title: Factors associated with the uptake of risk-reducing surgeries among Hispanic women at high risk of breast and ovarian cancer

Yanin Chavarri-Guerra1,2, Kai Yang3, Ian Komenaka4, Sandra Brown5, Azucena Del Toro Valero6, Pamela Mora Alférez5, Paul Duncan7, Yenni Rodríguez6, Pamela Ganschow9, Annette Campbell-Fontaine10, Gary Unzeitig12, Darling Horcasitas10, Nancy R Feldman11, Thomas Slavin2, Bita Nehoray7, Nancy Guerrero-Llamas2, Rosa Mejia2, Kathleen Blazer9 and Jeffrey Weitzel2. 1Instituto Nacional de Ciencias Médicas y Nutrición, Salvador Zubirán, Mexico, Mexico; 2City of Hope, Duarte, CA; 3St. Joseph Hospital and Mission Hospital, Orange, CA; 4Universidad de Guadalajara, Guadalajara, Mexico; 5Instituto Nacional de Enfermedades Neoplásicas, Lima, Peru; 6Clinica del Country, Bogota, Colombia; 7Hematology Oncology Associates, Albuquerque, NM; 8Maricopa Medical Center, Phoenix, AZ; 9Cook County Health and Hospitals Systems, Chicago, IL; 10New Mexico Cancer Center, NM; 11University of California Los Angeles Olive View Medical Center, Los Angeles, CA and 12Laredo Breast Care, Laredo, TX.

Body: Background: BRCA mutation carriers have an increased lifetime risk of breast and ovarian cancer. Although risk-reducing surgeries have documented utility, social factors, cultural beliefs and access to healthcare may be barriers among the Hispanic population to the uptake of risk-reducing practices. We aimed to describe the uptake of risk-reducing mastectomy (RRM) and risk-reducing salpingo-oophorectomy (RRSO) among Hispanic women referred for genetic cancer risk assessment (GCRA) and to identify factors affecting that uptake.

Methods: Hispanic women from the US and Latin America (LatAm) (Mexico, Colombia, Peru and Puerto Rico) referred for GCRA and enrolled in the Clinical Cancer Genomics Community Research Network (CCGCRN) registry from 1997 to 2016 were included. Demographic characteristics and data regarding risk-reducing surgeries were obtained from chart reviews and patient reported follow-up questionnaires. Data was analyzed using Fisher's test and x² statistics with a two-sided p value of <0.05 considered significant. An adjusted logistic regression model including a set of significant factors was constructed in order to predict the likelihood of undergoing risk-reducing surgery.

Results: Information about risk-reducing surgery status was collected on 1,784 Hispanic women referred for GCRA. Median age was 44.6 years (range 19-98), 84% (n=1506) had a personal history of any type of cancer and 18% (n=323) were BRCA carriers. Median follow-up was 2.6 years. 389 (22%) women had RRM, 167 (17%) had RRSO and 64 (4%) had both surgeries. RRM and RRSO occurred 32 days (SD 983.8) and 228 days (SD 1050.9) after GCRA, respectively. Among BRCA carriers, the rates of RRM and RRSO were 39.42% (n = 123) and 42.66% (n = 93), respectively. The frequency of RRM and RRSO among Hispanic BRCA carriers living in the US was higher than among those living in LatAm (53 vs. 29%, p < 0.01) and (67 vs 21.5%, p < 0.01), respectively. On multivariate analysis, the following factors were associated with a higher likelihood of undergoing RRM: BRCA positive status (HR 5.4, p< 0.01), personal history of breast cancer (HR 6.05, p < 0.01), living in the US (HR 6.53, p< 0.01) and one or more previous pregnancies (HR 1.42, p< 0.01). Educational level, marital status and age were not associated with a likelihood of undergoing RRM. BRCA positive status (HR 12.41, p< 0.01), personal history of breast cancer (HR 6.05, p < 0.01), living in the US (HR 6.53, p< 0.01) and one or more previous pregnancies (HR 1.42, p< 0.01). Educational level, marital status and family history of cancer were not associated with a higher likelihood of undergoing RRSO.

Conclusions: Our results suggest that the rate of RRM and RRSO among Hispanic women is lower than those reported in previous studies in other populations. Hispanic women living in US, BRCA carriers, and women with a personal history of cancer were more likely to have risk-reducing surgeries, which may be a reflection of disparities in access to care between countries and fragmented health systems, as well as of knowledge and cultural barriers. New strategies are warranted in order to improve the uptake of risk-reducing strategies among Hispanic women at an increased risk of cancer.
Body: Purpose/Objectives: Invasive micropapillary carcinoma (IMPC) is an uncommon variant of breast cancer, accounting for <2% of all cases. Previous studies demonstrated that this subtype often presents with hormone receptor (HR) positive disease, resulting in survival outcomes similar to invasive ductal carcinoma. However, many of these studies were conducted prior to the availability of HER2 testing. In this study, we aim to determine the impact of molecular marker status (including HER2 status) on survival outcomes of invasive micropapillary carcinoma.

Materials/Methods: The National Cancer Data Base (NCDB) was used to retrieve patients with biopsy-proven IMPC diagnosed from 2007-2012. Only patients with known estrogen receptor (ER) status, progesterone receptor (PR) status and HER2 status were included in the analysis. Cox multivariate regression was used to determine prognostic factors.

Results: Overall, 865 patients met inclusion criteria. The median follow-up was 2.5 years. Out of all patients, 651 (75.3%) had HR+, HER2 negative disease, 128 (14.8%) had HR+, HER2+ disease, 41 (4.7%) had HR negative, HER2+ disease, and 45 (5.2%) had triple negative disease. Patients with triple negative disease were more likely to have poorly differentiated/undifferentiated histology (66.7%), lymphovascular invasion (73.3%), stage 3 disease (37.8%), undergone mastectomy (68.9%), and positive surgical margins (15.6%). On Cox multivariate regression, those with triple negative disease had worse overall survival (hazard ratio 7.28, \( P < 0.001 \)). Other prognostic factors associated with worse overall survival included African American descent (hazard ratio 2.24, \( P = 0.018 \)), comorbidity score of 1 (hazard ratio 2.50, \( P = 0.011 \)), comorbidity score \( \geq 2 \) (hazard ratio 3.27, \( P = 0.06 \)), and \( \geq 3 \) positive lymph nodes (hazard ratio 3.23, \( P = 0.007 \)).

Conclusions: Similar to invasive ductal carcinoma, triple negative disease in invasive micropapillary carcinoma results in worse survival outcomes. This is the largest and first study to characterize molecular status (including HER2 status) in IMPC patients, as well as its impact on survival outcomes.
Title: Triple negative apocrine carcinomas as a distinct subtype of triple negative breast cancer: A case-control study

Icro Meattini1, Donato Pezzulla1, Giulio Alberto Carta1, Carlotta Becherini1, Marco Perna1, Roberta Grassi1, Pietro Garlatti1, Isacco Desideri1, Vieri Scotti1, Marco Bernini1, Luis Jose Sanchez1, Lorenzo Orzalesi1, Jacopo Nori1, Simonetta Bianchi1 and Lorenzo Livi1.

Azienda Ospedaliero Universitaria Careggi - University of Florence, Florence, Italy.

Body: Introduction. Invasive apocrine carcinoma of the breast is a rare type of breast cancer (BC), pure apocrine carcinoma constitutes <1% of all BC. Mammary apocrine epithelium has a characteristic steroid receptor profile that is negative for full length estrogen receptor-alpha and progesterone receptor and is androgen receptor positive. Conflicting data are available on the outcome of this type of disease: few studies reported significantly different prognosis of triple negative (TN) apocrine carcinomas when compared to most non-apocrine triple negative (NA-TN) tumors. The aim of this study is to report our long-term experience in a single-center series of TN apocrine tumors.

Methods. We analyzed clinical and pathological features of a series of TN apocrine carcinomas treated at our Centre in a 15-year period. Clinical and pathological characteristics and outcomes have been compared with a control series of NA-TN tumors treated during the same follow up period. Local relapse-free survival (LRFS), distant metastases-free survival (DMFS), and overall survival (OS) have been evaluated and compared between groups of patients.

Results. Forty-five TN apocrine carcinomas were analyzed. The mean age at diagnosis was 60 years (range 34-83 years). The proportions of apocrine tumor grades varied, with G1 being seen in 6.8% of patients, G2 in 51.1%, and G3 in 40.1%. The majority of apocrine carcinomas had small tumor size (T1: 72.7%; T2: 27.3%), and negative axillary nodal status (66.7%). The series was compared to a homogenous control group of 45 NA-TN patients. The mean age was 54 years (range 32-79 years), affected by high grade (G3: 53.7%), small tumor size (T1: 87.5%; T2: 12.5%), and mostly negative axillary nodal status (82.9%). LRFS in the apocrine group was 85% and 78% at 5- and 10-year, respectively. LRFS in the NA-TN group was 90% and 79% at 5- and 10-year, respectively. No difference was evidenced between groups (HR 1.44 95%CI 0.62-3.79; p=0.39). DMFS in the apocrine group was 85% and 85% at 5- and 10-years, respectively. DMFS in the NA-TN group was 85% and 75% at 5- and 10-year, respectively. DMFS was significantly better in the apocrine group (HR 0.69 95%CI 0.28-1.62). OS in the apocrine group was 86% and 83% at 5- and 10-year, respectively. OS in the NA-TN group was 86% and 63% at 5- and 10-years, respectively. OS was significantly better in the apocrine group (HR 0.57 95%CI 0.26-1.18).

Conclusions. Apocrine carcinomas represent a clinic-pathological distinct group of triple-negative BC, characterized by significantly more favorable clinical prognosis in terms of long-term disease-related morbidity or mortality when compared to NA-TN tumors.
Title: Identification of ESR1 mutation in breast cancers using targeted ultra-deep sequencing data analysis

Ji-Yeon Kim¹, Kyunghee Park², Woong-Yang Park², Seok Jin Nam¹, Seok Won Kim¹, Jeong Eon Lee¹, Se Kyung Lee¹, Hae Hyun Jung³, Jong Han Yu¹, Jin Seok Ahn¹, Young-Hyuck Im¹ and Yeon Hee Park¹. ¹Samsung Medical Center, Seoul, Korea; ²Samsung Genome Institute, Seoul, Korea and ³Samsung Advanced Institute for Health Sciences and Technology, Seoul, Korea.

Body: Introduction: Estrogen Receptor 1 (ESR1) gene encodes an estrogen receptor, which regulates cell proliferation and promotes tumor progression in estrogen receptor (ER)-positive breast cancer (BC). Therefore, endocrine therapy that inhibiting ER downstream signal, is the most effective treatment strategy in ER-positive BC. However, about 25% of patients with primary disease and almost all patients with metastases will present with or eventually develop endocrine resistance. And genetic alteration of ESR1 is now identified as the endocrine resistance mechanism. However, a few data from clinical trials or public data base exists and could not reflect real world clinic. Therefore, we aimed to identify the frequency and type of ESR1 genetic alterations in BCs through this large scaled study.

Methods: We performed targeted ultra-deep sequencing (CancerSCAN™) using BC tissue specimens. This sequencing was covered entire coding area of ESR1 gene and also detected copy number alteration and translocation of ESR1.

Results: Targeted ultra-deep sequencing of ESR1 was performed using 618 BC tissues. Of 618 tissue samples, 253 (40.9%) were MBCs, 362 (58.6%) were early BCs (EBCs) and 3 were not identified. In terms of subtypes, 220 ER-positive BCs, 122 ER-positive and HER2-positive BCs, 119 HER2-positive and 153 triple-negative BCs (TNBCs) were included. BCs from patients under 40 year-old were 277 (44.8%) (Median: 43.0, range: 23.5 -75.6). ESR1 genetic alterations were identified in 21 BCs (5 EBCs and 16 MBCs). In EBCs, 3 cases were observed in TNBCs and 2 cases were in ER-positive BCs (2.6% and 1.2%, respectively). All five EBC were treatment naïve status. Of 16 cases of ESR1 alterations in MBCs, 10 cases of ESR1 alterations were detected in ER-positive BCs (17.6%), 5cases in ER and HER2-positive BCs(6.7%) and 1 in HER2-positive BCs (1.2%). All ER-positive MBCs were treated with more than one line of endocrine therapy. Most commonly detected genetic alteration was single nucleotide variant (SNV) (15 of 21, 71.4%). Thirteen were in ligand binding domain and two cases occurred in activation function-1 (AF-1) domain (P79A and G145S). D538G and V392I were most frequently mutated loci followed by Y537N (3, 3 and 2 cases, respectively) and only metastatic ER-positive BCs harbored ESR1 activating mutation. Four copy number (CN) amplification in 2 ER-positive and 2 ER and HER2-positive BCs, one CN deletion in TNBC and one ESR1 fusion in ER and HER2-positive BC were also detected (19.0%, 4.8% and 4.8%, respectively). In frame ESR1 fusion was occurred between ESR1 and NPHS1 genes.

Conclusion: In this experimental study, ESR1 genetic alterations were frequently detected in ER-positive MBC but ER-negative or EBC also harbored. The type of genetic alterations varied including SNVs, CN alterations and translocation and ESR1-NPHS1 fusion is the novel genetic alteration that has not been reported. To identify the role of ESR1 genetic alteration in ER-negative BCs and novel translocation, further functional validation would be warranted (Clinical trials.gov Number :NCT02591966).
Title: Meta-analysis of ESR1 mutation prevalence in metastatic breast cancer

Pablo Mandó¹, Manglio M Rizzo¹, Constanza Perez de la Puente¹, Maria V Costanzo¹, Adrian Nervo¹, Jorge Nadal¹, Federico Colo¹, Carlos M Loza¹, Jose Loza¹, Veronica Fabiano¹, Carolina Ponce¹ and Reinaldo Chacon¹. ¹Instituto Alexander Fleming, Ciudad de Buenos Aires, Argentina.

Body: Introduction: ESR1 mutations were described for the first time in 1996 when different tyrosine 537 mutations were found to confer constitutive activation of the receptor, describing this region as a key factor in the ligand regulation of ER transcriptional activity. However, due to the low prevalence of this mutation in primary breast tumors its clinical significance maintained unknown. With the advent of large scale genomic analysis, a new understanding of breast cancer molecular characteristics has gained relevance. The low prevalence of ESR1 mutations in primary breast cancer has been confirmed but mutations in metastatic ER-positive breast cancers has been proved to be a completely different scenario. Nevertheless, information regarding real prevalence of ESR1 mutation in metastatic breast cancer is not known as selection of patients and molecular technique used are heterogeneous.

Sources: Search was carried by corresponding clinical oncologists of the Breast Cancer Unit of Alexander Fleming Institute. In March 2017, key words “ESR1 mutations”, “Estrogen receptor mutations” and “Breast cancer” were used as search strategy for the present meta-analysis in PubMed. Furthermore, abstracts from congress presentations were analyzed and hand searching from reference list of obtained articles was executed. Online search retrieved 60 articles published, 3 abstracts related were found and 3 further studies were detected by hand search. Articles were excluded if they only included primary tumors and not metastatic cases and if they were undertaken before 2000 due to important technical differences of mutation detection, including finally 23 cohorts.

Study Selection: Studies considered were prospective or retrospective cohorts of metastatic breast cancer patients with mutation analysis of tissue or circulating DNA. A data form was used by the primary reviewer to extract equivalent information from each article. Information extracted included population sampled, prevalence estimates, clinical characteristics of cohort, sample analyzed and technical procedure for mutation detection. A second reviewer blinded to the primary reviewer’s decisions checked the article selection and data extraction. Any differences of opinion were discussed, and a third reviewer was available to arbitrate any issues.

Meta-analysis was undertaken using a random-effects model conducted using the metaprop function in Meta package of R studio Version 1.0.136 (© 2009-2016 RStudio, Inc.). PRISMA guidelines were followed in conducting and reporting the results.

Results and Discussion: Results show a prevalence of ESR1 mutation of 24% (CI95% 19-30%) in the 3607 patients included. Nevertheless, important heterogeneity (I² =90%) is observed due to great differences in the articles published of this topic. This heterogeneity is attributed to the type of cohorts presented, the selection of patients, the technique used and type of sample studied, but another source of heterogeneity must be present as it still persists after grouping studies according to this variables. The importance of this analysis resides in the fact that it is the most complete information of the prevalence of this mutation that may have future importance in therapeutic decisions in metastatic breast cancer.
Title: Provider characteristics and receipt of oncostype Dx testing in women diagnosed with early stage breast cancer using SEER-Medicare data

Michaela A Dinan¹, Lauren E Wilson², Melissa Greiner² and Craig E Pollack³. ¹Duke Cancer Institute, Durham, NC; ²Duke University Medical Center, Durham, NC and ³Johns Hopkins Medicine, Baltimore, MD.

Body: Background: Oncotype DX (ODX) genomic testing to evaluate recurrence risk and benefit of adjuvant chemotherapy in patients with ER-positive, node-negative breast cancers was approved for Medicare reimbursement in 2006. We previously examined patient-level factors associated with utilization of ODX testing from 2005-2009 in the SEER-Medicare population; ODX testing occurred most frequently in patients with ER+, node negative disease, with 80% of all tests occurring in patients aged 66-75. In our current study, we examined potential provider factors associated with patient-level ODX testing from 2008 to 2012.

Methods: Using a retrospective cohort design, we identified all individuals who had a SEER diagnosis of breast cancer from 2008-2011 and were enrolled in fee-for-service Medicare parts A and B for one year before and one year after diagnosis. We limited our analysis to individuals who had surgical resection of their breast tumor within 4 months of diagnosis and had a breast tumor which was ER+, invasive, and non-metastatic to capture the eligible patient population. Using Medicare claims data linked with the AMA physician dataset (which includes AMA members and non-members), we identified physician characteristics of the primary breast surgeon and medical oncologist including specialty, gender, years in practice, case volume, utilization of chemotherapy, and whether they serve rural populations. For patients with an ODX test, we used the identification on the claim to link to the performing provider. We examined the associations between provider characteristics and patient receipt of ODX testing using unadjusted and adjusted logistic regression models. Adjusted models included patient demographic and clinical characteristics.

Results: We identified 24,463 eligible breast cancer patients who received their care from 3172 primary surgeons and 2475 medical oncologists. Of 4124 ODX tests ordered for patients in the study, 70% were ordered by the assigned medical oncologist and 16% were ordered by the breast surgeon. In multivariable regression models, multiple physician characteristics were associated with receipt of ODX testing including having an assigned medical oncologist (OR 2.77, 95% CI 2.00-3.82), having a surgeon with a specialty of surgical oncology (OR 1.20, 95% CI 1.09-1.31), having a female medical oncologist (OR 1.10 95% CI 1.02-1.20). Having a medical oncologist with ≥5 years in practice was associated with lower odds of testing (OR 0.83 95% CI 0.76-0.92). Breast surgery performed at an academic hospital was associated with higher odds of ODX testing (OR 1.11 95% CI 1.02-1.20).

Conclusion: The majority of ODX testing for indicated breast cancer patients is ordered by medical oncologists, though surgeons and physicians of other specialties also order the tests in practice. Physician characteristics including gender and time in practice appear to affect a patient's likelihood of receiving ODX testing, creating opportunities for targeting interventions to help women with breast cancer receive optimal care.
Title: Ki67 cut offs and oncotype DX recurrence score in early breast cancer

Fernando Namuche¹, Rossana Ruiz¹, Rossana Ruiz¹, Claudio Flores¹, Henry L Gomez¹,² and Alfredo Aguilar¹. ¹Oncosalud, Lima, Peru and ²INEN, Lima, Peru.

Body: Background: The gene expression profiling assay OncotypeDx (ODX) predicts the likelihood of estrogen receptor (ER) positive breast cancer (BC) recurrence and assesses the likely benefit from both hormonal therapy and chemotherapy. Many clinical scores that estimate the risk category of ODX are being tested. Ki67 is frequently incorporated into these assessments, although there is no standard cut-off for its use.

Methods: We retrospectively reviewed the electronic medical records of 190 patients with early stage ER+ BC for whom ODX recurrence score (RS) was available. Patients were diagnosed and treated at a specialized cancer center between 2014 and 2016. Our objective was to find out the degree to which an optimal ki67 cut-off correlates with ODX risk category. We also aim to determine an association between classical clinicopathological variables (St. Gallen (SG) 2015) could predict ODX risk category. Chi square test was used.

Results: The characteristics of patients according to ODX risk category are shown in Table 2. Mean age at diagnosis was 59 years (range 28-89). Mean tumor diameter was 15mm, 84.2% were intermediate grade and 4.7% patients had lymph node involvement. Mean expression of ER, PR and Ki67 were 87%, 53% and 22%, respectively. According to ODX 62.1% patients were low risk, 30.5% were intermediate risk and 7.4% were high risk. An overall concordance of 46.8% (73/190) was found between SG 2015 and the risk category of ODX (75.7% for low, 33.3% for intermediate and 23.9% for high RS). When changing SG Ki67 cutoffs to ≤20% (for low Ki67) and ≥30% (for high Ki67), an overall concordance of 56.3% (107/190) was found (69.6% for low, 47.3% for intermediate and 23.9% for high RS) and this was statistically significant (p=0.00) (Table 3).

Conclusion: In our population there is no a direct correlation between classical clinicopathological variables and ODX score. Despite being a specialized center, the utility of classical clinicopathological variables for predicting ODX risk category is limited.
Title: A functional single nucleotide polymorphisms in ABCC11, rs17822931, is associated with the risk of breast cancer in Japanese

Junko Ishiguro¹, Hidemi Ito¹, Megumi Tsukamo², Hiroji Iwata³, Hiroshi Nakagawa² and Keitaro Matsuo¹. ¹Aichi Cancer Center Research Institute, Nagoya, Japan; ²College of Bioscience and Biotechnology, Chubu University, Kasugai, Japan and ³Aichi Cancer Center Hospital, Nagoya, Japan.

Body: Background: ATP binding cassette (ABC) transporters are members of membrane protein of transporters, genetic variations of which may influence their activity. ABCC11, a member of ABC transporters, has been known to export steroid sulfates such as E2-17bG and E3S that are precursor of active estradiol. Therefore, rs17822931, a functional single nucleotide polymorphisms (SNP) in the ABCC11, may play a role in carcinogenesis of breast cancer (BC) via estrogen exposure. Three previous studies about the association of this polymorphism with BC risk reported inconsistent results. In this study, we aimed to evaluate the association between ABCC11 rs17822931 and BC risk in a Japanese population.

Subjects and Methods: We conducted a case-control study in 697 BC patients and 1394 age- and menopausal status-matched controls within the framework of the Hospital-based Epidemiological Research Program at Aichi Cancer Center II (HERPACC II). The odds ratio (OR) and 95 % confidence interval (CI) were calculated using the conditional logistic model adjusted for potential confounders to evaluate the association. We evaluated heterogeneity by BC subtype using Cochran's Q test, and that by estrogen exposure by multiplicative assumption in the conditional logistic regression model, which included an interaction term for genotype and estrogen exposure variable.

Results: Compared with A-allele, G allele was inversely associated with BC risk (a per allele OR=0.76, 95% CI, 0.61–0.94, \( P = 0.012 \)). In the stratified analysis, we found that this association was observed only in estrogen receptor (ER) positive BC. A per-allele OR for ER positive BC was 0.65 (95% CI 0.49–0.86) while that for ER negative BC was 1.10 (95% CI 0.77-1.72). We found a statistically significant heterogeneity by ER status (\( P \) for interaction = 0.047). We did not observe any heterogeneity in the stratified analysis by other tumor characteristics. When stratifying by factors related with estrogen exposure, we found that this polymorphism had a different impact on BC risk by levels of estrogen exposure. Compared to women with low exposure to estrogen, those with high exposure had a stronger impact of this polymorphism. Per allele ORs for women who had ever and never breastfeeding were 0.82 (95% CI, 0.65-1.04) and 0.45 (0.26-0.79), respectively. We found suggestive heterogeneity between rs17822931 and history of breastfeeding on BC risk (\( P \) for interaction = 0.093). Interestingly, we found heterogeneous impact of rs17822931 by the levels of soy food consumption which is well known that may exert their effects as estrogen antagonists (\( P=0.005 \)). We observed that ORs for >50, 30-49 and <30g/day of soy intake were 0.98 (0.65-1.46), 0.82 (0.59-1.14) and 0.45 (0.29-0.69), respectively.

Conclusion: Current case-control study showed the significant association between rs17822931 and the risk of BC, especially ER-positive BC, in Japanese women. Compared to women with low estrogen efflux activity with A allele, those with high efflux activity with G allele may have a lower risk of BC, especially in women with high estrogen exposure. To the best of our knowledge, this is the first study reporting the association between rs17822931 on ABCC11 and risk of BC considering levels of estrogen exposure.
Taofik O Oyekunle, Samantha M Thomas, Rachel A Greenup, Terry Hyslop and Kimberly Blackwell. 1Duke Cancer Institute, Durham, NC and 2Duke University Medical Center, Durham, NC.

Body: Introduction:
Breast cancer (BC) is the most commonly diagnosed cancer among women in the United States, but less than 5% of women develop BC before age 40. We sought to determine the trend over time in incidence and survival rates, and pathologic features of Non-Hispanic White (W), Non-Hispanic Black (B) and Hispanic (HIS) women (<40 years) with invasive breast cancer.

Methods:
Women <40 years old diagnosed with invasive BC were identified from the SEER 13 registry. Patients were stratified by year of diagnosis (1992-2014), race/ethnicity (W, B, and HIS) and pathologic features (stage, grade, ER and PR status). Age-adjusted incidence rates and 5- and 10-year disease-specific survival (DSS) rates were calculated. Incidence rate ratios (IRRs) were estimated by race/ethnicity group and pathologic features to express relative risk of BC incidence. Temporal trends of incidence rates (1992-2014), 5- (1992-2009) and 10-year (1992-2004) DSS rates were assessed as average annual percentage change (AAPC) using a joinpoint model. Survival estimates were calculated using the Kaplan-Meier method and Log-rank tests were used to test for differences in DSS among the race/ethnicity groups.

Results:
A total of 28,686 patients were included in this analysis: 64.1% W, 16.6% B, and 19.3% HIS. Overall, young B women had a higher incidence and worse survival than W women. Tumors with poor prognostic features (stage IV, grade III, ER- and PR-) were more common than those with better prognosis (stage 1, grade 1, ER+ and PR+, respectively) among young B women compared to W and HIS women. Young B women had worse 5- and 10-year DSS compared to their W and HIS counterparts (all p<0.001).

Trends of incidence and mortality rates by race/ethnicity and pathologic features.

<table>
<thead>
<tr>
<th></th>
<th>W</th>
<th>B</th>
<th>HIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall vs HIS</td>
<td>1.38 (1.34-1.42)</td>
<td>1.74 (1.69-1.81)</td>
<td>reference</td>
</tr>
<tr>
<td>Stage 4 vs 1</td>
<td>0.13 (0.12-0.14)</td>
<td>0.41 (0.36-0.46)</td>
<td>0.26 (0.23-0.30)</td>
</tr>
<tr>
<td>Grade 3 vs 1</td>
<td>7.43 (7.02-7.89)</td>
<td>13.82 (11.99-16.00)</td>
<td>10.40 (9.22-11.76)</td>
</tr>
<tr>
<td>ER- vs +</td>
<td>0.55 (0.53-0.56)</td>
<td>0.83 (0.78-0.88)</td>
<td>0.64 (0.60-0.66)</td>
</tr>
<tr>
<td>PR- vs +</td>
<td>0.71 (0.69-0.73)</td>
<td>1.13 (1.06-1.20)</td>
<td>0.85 (0.80-0.91)</td>
</tr>
<tr>
<td>DSS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-yr</td>
<td>86.7 (86.1-87.2)</td>
<td>72.9 (71.5-74.3)</td>
<td>80.6 (79.4-81.8)</td>
</tr>
<tr>
<td>10-yr</td>
<td>78.0 (77.3-78.7)</td>
<td>63.2 (61.5-64.8)</td>
<td>71.4 (69.9-72.9)</td>
</tr>
<tr>
<td>AAPC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>0.8*</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Stage 4</td>
<td>4.3*</td>
<td>4.4*</td>
<td>3.0*</td>
</tr>
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<td>Grade 3</td>
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<td>1.1</td>
<td>1.0*</td>
</tr>
<tr>
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<td>-0.5</td>
<td>0.2</td>
</tr>
<tr>
<td>PR-</td>
<td>1.1*</td>
<td>1.2*</td>
<td>1.9*</td>
</tr>
<tr>
<td>DSS</td>
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<tr>
<td></td>
<td>5-yr</td>
<td>10-yr</td>
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<td></td>
<td>0.6*</td>
<td>0.9*</td>
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<td></td>
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<td>1.1*</td>
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</tr>
</tbody>
</table>

*AAPC is statistically different from zero (p<0.05).

**Conclusion:**
The incidence of higher stage and PR- tumors is increasing at a faster rate in young black women when compared to whites. Although the incidence of BC is increasing over time for young white women and not young black women, disparities still exist in overall incidence. Similarly, although DSS is increasing at higher rates for black and Hispanic women compared to whites, large survival disparities still exist. Improvements have been made over time, but more work needs to be done to determine which factors are associated with these disparities and how to close the gap in survival.
2017 San Antonio Breast Cancer Symposium

Publication Number: P6-10-02

Title: Black race is associated with worse distant relapse-free survival in breast cancer patients treated with neoadjuvant compared to adjuvant systemic chemotherapy

Jessica M Pastoriza¹, George S Karagiannis²,³, Xionan Xue², Juan Lin², John S Condeelis², Joseph A Sparanno¹, Thomas E Rohan² and Maja H Oktay¹,². ¹Montefiore Medical Center, Bronx, NY; ²Albert Einstein College of Medicine, Bronx, NY and ³Integrated Imaging Program, Albert Einstein College of Medicine, Bronx, NY.

Body: Background: Compared to white women, black women with operable breast cancer treated with primary surgical therapy and adjuvant or neoadjuvant systemic chemotherapy have higher recurrence rates and breast cancer mortality. Large randomized prospective studies did not find significant differences in distant-recurrence free survival (DRFS) and overall survival (OS) between breast cancer patients treated in the adjuvant and neoadjuvant setting for predominantly white populations. However, data indicating that neoadjuvant treatment is equivalent to adjuvant treatment for black breast cancer patients are missing. Here, we first examined racial differences in DRFS among breast cancer patients treated in the neoadjuvant setting at Einstein-Montefiore Center for Cancer Care (EMCCC) in the Bronx, and then investigated if DRFS in black patients treated in the neoadjuvant setting is comparable to DRFS in the adjuvant setting.

Methods: We evaluated DRFS in 241 racially diverse patients with localized or regionally advanced breast cancer treated with neoadjuvant chemotherapy between January 2000 and December 2016. In addition, we evaluated DRFS in 474 white and 701 black patients with localized or regionally advanced breast cancer treated with systemic adjuvant (432 whites, 596 blacks) or neoadjuvant (42 whites, 105 blacks) chemotherapy. Using multivariate Cox proportional hazard models, we generated hazard ratios (HR) and 95% confidence intervals (95%CI) for risk of distant recurrence, with adjustment for age (<50 vs >/50 years), stage (I/II vs III), estrogen receptor (ER) status (+ vs -), HER2/neu overexpression (+ vs. -/equivocal/unknown), triple negative (TN) status (yes vs no), and type of systemic chemotherapy (adjuvant vs. neoadjuvant).

Results: Black patients treated with neoadjuvant systemic chemotherapy had significantly worse DRFS than white patients (HR=2.29; 95%CI=1.02-5.15, p=0.04). DRFS in non-black Hispanics and patients from racial backgrounds other than Hispanic or black compared to whites was not statistically different. Neoadjuvant chemotherapy was associated with worse DRFS compared to adjuvant chemotherapy in black (HR=3.72; 95%CI=4.03-5.81; p=<0.0001), but not in white women.

Conclusion: Black patients with localized breast cancer treated with systemic neoadjuvant chemotherapy not only have inferior DRFS compared to white patients, but also worse DRFS when compared to black patients treated with adjuvant chemotherapy, after adjustment for clinical and pathological covariates. This observation needs to be confirmed in further prospective studies and biologic factors contributing to this finding need to be evaluated.
Title: The association between type-2 diabetes and risk of mortality after invasive breast cancer among Hispanic and non-Hispanic white women from New Mexico

Avonne E Connor1, Kala Visvanathan1, Stephanie D Boone2, Kathy B Baumgartner2 and Richard N Baumgartner2. 1Johns Hopkins Bloomberg School of Public Health, Baltimore, MD and 2University of Louisville, Louisville, KY.

Body: Background: Evidence suggests that the presence of Type-2 diabetes at the time of breast cancer diagnosis adversely affects survival independent of breast cancer stage, grade, and tumor phenotype. Few of these epidemiological studies have included Hispanic breast cancer survivors in whom diabetes and obesity are prevalent.

Objective: We examined the association between self-reported diabetes history, breast cancer-specific and all-cause mortality among Hispanic and non-Hispanic white (NHW) women diagnosed with breast cancer (stages I-IIIa) from the New Mexico Health, Eating, Activity, and Lifestyle cohort.

Methods: A total of 399 breast cancer survivors (96 Hispanic, 303 NHW) contributed data for the present study. Women with breast cancer diagnosed between July 1996 and March 1999 were ascertained through the National Cancer Institute's Surveillance Epidemiology and End Results (SEER) registry in New Mexico. Baseline demographic characteristics and breast cancer risk factors were collected approximately 5 months post diagnosis by trained interviewers. Hazard ratios (HR) and 95% confidence intervals (CI) were calculated using multivariable Cox proportional hazards regression models. Models were further stratified by ethnicity.

Results: After a median follow-up time of 13.5 years from baseline interview to death, a total of 134 deaths occurred. The prevalence of diabetes did not significantly differ by ethnicity in our study; 11.5% of Hispanics reported having diabetes compared to 7.5% of NHW women. While a history of diabetes was associated with older age at breast cancer diagnosis (p=0.001), higher percent body fat (p=0.01), higher body mass index (p<0.001), and increased waist-hip ratio (p< 0.001) compared to non-diabetics, no significant differences were observed between diabetics and non-diabetics for breast cancer stage, grade, tumor phenotype, or receipt of breast cancer treatment. In multivariable models, diabetes was associated with increased risk of all-cause mortality overall (HR, 2.10; 95% CI 1.24-3.55), with a significant association only observed among Hispanic women (HR, 3.07; 95% CI 1.05-8.94) when compared to NHW women (HR, 1.66; 95% CI 0.86-3.24). The interaction between ethnicity and diabetes was not statistically significant for all-cause mortality. Diabetes also was significantly associated with increased risk of breast cancer-specific mortality (HR, 2.89; 95% CI 1.27-6.60); however results were not statistically significant by ethnicity.

Conclusions: Overall, diabetes significantly increased risk of all-cause mortality among women diagnosed with invasive BC from our study, particularly among Hispanic women. Diabetes was also found to be a significant prognostic factor for breast-cancer specific mortality. Future studies utilizing this data will be conducted to further evaluate the association between diabetes, glycemic control and breast cancer-specific mortality in Hispanic and NHW women.
2017 San Antonio Breast Cancer Symposium

Publication Number: P6-10-04

Title: “Disparities in breast cancer: A multi-institutional comparative analysis focusing on American Hispanics”

Salman Otoukesh¹, Zeina Nahleh², Hamid R Mirshahidi³, Anhony L Nguyen⁴, Gehan Botrus⁵, Nabeel Badri⁶, Nabih Diab⁷, Andres Alvarado⁸, Luis A Sanchez⁹ and Alok Dwivedi¹⁰. ¹Loma Linda University, Loma Linda, CA; ²Texas Tech University Health Sciences Center (TTUHSC), El Paso, TX; ³Loma Linda University, Loma Linda, CA; ⁴Loma Linda University, Loma Linda, CA; ⁵Texas Tech University Health Sciences Center (TTUHSC), El Paso, TX; ⁶Texas Tech University Health Sciences Center (TTUHSC), El Paso, TX; ⁷Texas Tech University Health Sciences Center (TTUHSC), El Paso, TX; ⁸Texas Tech University Health Sciences Center (TTUHSC), El Paso, TX; ⁹Texas Tech University Health Sciences Center (TTUHSC), El Paso, TX and ¹⁰Texas Tech University Health Sciences Center (TTUHSC), El Paso, TX.

Body: Background: Breast cancer (BC) is the leading cause of cancer death in Hispanic/Latina women nationwide. Hispanic women are more likely to be presented with advanced disease and might have adverse prognosis. Further, the Hispanics of Mexican-American origin might reflect different clinico-pathological characteristics as opposed to other Hispanics and ethnic groups. No previous largest studies comprised with Hispanics of Mexican-American origin explored tumor characteristics and compared to other ethnic groups. Thus, the aim of this study was to describe the clinico- pathological characteristics and disparities in breast cancer in this minority group at two tertiary care University- based medical centers in 2 states with a large Hispanic presence.

Methods: After IRB approval, cancer registry was used to analyze the variables of 3,441 patients with breast cancer diagnosed and treated consecutively at two large tertiary University based medical centers in El Paso, TX and Loma Linda, CA between 2005-2015. Unadjusted and adjusted associations of race/ethnicity with cancer stage, hormone receptor status and treatment option were investigated, as well as comparison to other ethnic groups.

Results: Overall 45.5% of the patients were Hispanic (n= 1566). Hispanics were more likely to be diagnosed at a younger age (57 years) compared to non-Hispanic (NH) whites, more likely to have invasive ductal carcinoma type (82.7%) & triple negative disease (17.1%, 95%CI: 15% to 19%). 58.8% of Hispanics (95%CI: 56% to 61%) have HR+ & HER2- as opposed to 71% in NH whites. In addition, Hispanic individuals presented with advanced stages (III and IV) of BC (25.3%, 95% CI: 23% to 28%) similar to African Americans (25.4%), and had a lower proportion of lumpectomy versus mastectomy compared to NH whites (50%) but similar to African Americans (50%). Hispanic patients had the highest prevalence of triple negative BC (17.1% in Hispanics Versus 13.9 % in African Americans, versus 8.5% in NH whites). Hispanics also had significantly higher relative risk of HER2+/HR - disease (RRR=1.77, p<0.0001) compared to NH whites with no difference in African Americans (RRR= 1.21, p=0.56).

Conclusions: This large multi-institutional study shows that Hispanics are diagnosed with breast cancer at a younger age, have a higher prevalence of triple negative and HER2 positive/HR- breast cancer, are diagnosed at more advanced stages of disease and undergo less lumpectomies compared to NH whites. Increased efforts geared toward early detection, improving awareness and access to health care is desperately needed in this rapidly increasing minority in the U.S.
Title: UPTAKE study - Uptake of preventive surgeries among Latinas with BRCA1/2 mutations

Filipa Lynce¹, Adriana Serrano², Sue Friedman³, Zeina Nahleh⁴, Julie Dutili⁵, Claudia Campos⁶, Charité Ricker⁷, Patricia Rodriguez⁸, Ysabel Duron⁹, Claudine Isaacs² and Kristi Graves². ¹Lombardi Comprehensive Cancer Center, MedStar Georgetown University Hospital, Washington, DC; ²Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC; ³Facing Our Risk of Cancer Empowered, Tampa, FL; ⁴Maroone Cancer Center, Cleveland Clinic Florida, Weston, FL; ⁵Ponce School of Medicine and Health Sciences, Ponce, Puerto Rico; ⁶Nueva Vida, Alexandria, VA; ⁷USC Norris Comprehensive Cancer Center, Los Angeles, CA; ⁸US Oncology, Arlington, VA and ⁹Latinas Contra Cancer, San Jose, CA.

Introduction: Germline testing for BRCA1/2 genes provides an opportunity to reduce mortality and morbidity by adopting appropriate risk reduction and screening options, in particular with risk-reducing bilateral salpingo-oophorectomy (BSO). There is a paucity of data on Latinas and prophylactic measures among BRCA1/2 carriers. Existing studies are limited either by the small number of Latinas, or limited to a specific geographic location. Factors related to decision making have also not been evaluated. Methods: The UPTAKE study is an observational study of Latinas with germline BRCA1/2 mutations. Subjects were recruited nationally and, by telephone interviews, reported uptake of prophylactic surgeries (BSO, bilateral mastectomy in unaffected women, and contralateral mastectomy in carriers with breast cancer (BC)). Women with ovarian cancer were ineligible. All women had to have been informed that they carried a deleterious BRCA1/2 mutation at least 1 year prior to completing the interview. The objectives of this study are: 1) to examine the rate of uptake of prophylactic surgeries; 2) identify acculturation and attitudinal factors related to decisions made and 3) examine relationships between primary language, receipt of genetic counseling (GC) and in which language it was provided and uptake of prophylactic surgeries. We plan to enroll 100 participants.

Results: As of 6/11/2017, 86 telephone interviews have been conducted. We anticipate that all 100 interviews will be completed by July 2017. 51.2% (44/86) of participants completed the interview in Spanish. Our population was diverse in terms of country of origin: 50.0% (43/86) were born in the US, 22.1% (19/86) in Mexico, 11.6% (10/86) in Puerto Rico, 4.6% (4/86) in El Salvador, 3.5% (3/86) in Ecuador and 8.1% (7/86) in other countries of Latin America. 30% (26/86) of the participants reported an annual household income inferior to $50,000. Only 26.7% (23/86) of women reported having a graduate degree. Approximately one quarter of participants were unemployed at the time of study participation (26.7%, 23/86). 34.9% (30/86) were unaffected and 62.8% (54/86) were affected with BC. 73.3% (63/86) of participants reported having received formal GC, of which only 28.6% (18/63) was conducted in Spanish. 66.3% (57/86) of women opted to undergo BSO and 58.1% (50/86) underwent prophylactic mastectomy. Being born outside the US and currently working were associated with higher uptake of BSO. Multivariate analysis will be performed once all interviews have been completed.

Conclusions: To our knowledge this is the largest study that evaluates uptake of prophylactic measures in Latinas known to be BRCA1/2 carriers. Our study included a heterogeneous group of participants in terms of country of origin, income and level of education including English knowledge. It was conducted across various academic and community centers in the country. The uptake of prophylactic surgeries among Latinas with germline BRCA mutations seems to be slightly lower than what has been reported in non-Hispanic whites (71-74%) but higher than in African Americans (32-50%). Results and factors associated with decision making will be updated once the total number of participants is enrolled.
Title: Racial differences in the characteristics and outcomes of young breast cancer patients: A national population-based study

James T McClain¹, Catalina Mosquera¹ and Mahvish Muzaffar¹. ¹East Carolina University, Greenville, NC.

Body:

Background:
Disparity in demographic characteristics as it relates to breast cancer outcomes is well-studied. However, studies evaluating racial differences exclusively among young patients are more limited. We sought to examine socioeconomic and clinical factors and their impact on outcomes in young patients, as well as to determine whether variation in outcomes changed over the 22-year study period.

Methods:
Using the Surveillance, Epidemiology, and End Results (SEER) database, we identified female patients aged 20-35 with invasive breast cancer diagnosed from 1990-2012. We performed univariate, multivariate and survival analysis. Variables included patient age, race, stage, receptor status, surgery type and year of diagnosis.

Results:
A total of 18,999 women were identified and analyzed. Mean age was 31.7 years. 31.2% were diagnosed between 1990-2000 while 68.7% were diagnosed between 2001-2012. 80.8% (15,364) of patients were white and 19.1% (3,635) were black. A higher percentage of blacks had stage III/IV disease (34% v 27%) and ≥ 4 positive nodes (19% v 16%) compared to whites. 54% of whites were ER receptor positive while 46% of blacks were ER receptor positive (p<0.0001). White patients were more likely to live in counties where ≤15% of households were below the poverty line (64% v 45%) and where ≤15% of the population had less than a high school education (35% v 28%) compared to blacks.

The overall 5-year disease specific survival (DSS) for the entire cohort was 82.5%. 5-year DSS was 84.4% for all white patients and 74.2% for all black patients (p<0.0001). 5-year DSS was 79.1% among all patients diagnosed from 1990-2000 and 84.2% among patients diagnosed from 2001-2012 (p<0.0001). While the 5-year DSS for white patients improved from 80.9% in 1990-2000 to 86.3% in 2001-2012 (p<0.0001), the 5-year DSS improvement for black patients from 1990-2000 to 2001-2012 did not reach statistical significance (71.3% vs 75.7 %, p=0.24).

Discussion:
Racial disparity among breast cancer patients is also an issue in young females, as young white patients have superior disease-specific survival compared to African-Americans collectively and in each time-period studied. Absolute disease-specific survival has improved from 1990-2000 to 2001-2012 for both races. However, the statistically significant difference in improvement of disease-specific survival seen among white patients was not demonstrated in African-American patients.
Continued attention to racial disparity in breast cancer outcomes is needed with additional studies examining potential differences in treatment, disease characteristics and biology, and accessibility to health care, with a particular focus on young cancer patients. With continued research, hopefully new treatment approaches will be developed to reduce this disparity.

5-Year Disease Specific Survival

<table>
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<tr>
<th>Time Period</th>
<th>5 year DSS</th>
<th>p value</th>
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<tbody>
<tr>
<td>White</td>
<td>Black</td>
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</tr>
<tr>
<td>1990-2000</td>
<td>80.9%</td>
<td>71.3%</td>
</tr>
<tr>
<td>2001-2012</td>
<td>86.3%</td>
<td>75.7%</td>
</tr>
<tr>
<td>p value</td>
<td>0.0001</td>
<td>0.24</td>
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</tbody>
</table>
## Survival by stage

<table>
<thead>
<tr>
<th></th>
<th>White</th>
<th>Black</th>
<th>p value</th>
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</thead>
<tbody>
<tr>
<td>Stage I</td>
<td>96.2%</td>
<td>94.9%</td>
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</tr>
<tr>
<td>Stage II</td>
<td>89.0%</td>
<td>83.5%</td>
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</tr>
<tr>
<td>Stage III</td>
<td>69.4%</td>
<td>57.4%</td>
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</tr>
<tr>
<td>Stage IV</td>
<td>34.6%</td>
<td>16.9%</td>
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</tbody>
</table>
2017 San Antonio Breast Cancer Symposium

Publication Number: P6-10-07

Title: Differences in genome-wide DNA methylation levels in breast milk by race and lactation duration

Brittny C Davis Lynn¹, Clara Bodelon¹, Ruth M Pfeiffer¹, Hannah P Yang¹, Howard Yang¹, Maxwell Lee¹, Peter W Laird², Mihaela Campan², Daniel J Weisenberger², Jeanne Murphy³, Mark E Sherman⁴, Eva P Browne⁵, Douglas L Anderton⁶, Kathleen F Arcaro⁶ and Gretchen L Gierach¹. ¹National Cancer Institute, Rockville, MD; ²University of Southern California, Los Angeles, CA; ³Patient Centered Outcomes Research Institution, Washington, DC; ⁴Mayo Clinic, Jacksonville, FL; ⁵University of Massachusetts at Amherst, Amherst, MA; ⁶University of South Carolina, Columbia, SC and ⁷Van Andel Research Institute, Grand Rapids, MI.

Body: Introduction: Identifying biomarkers of breast cancer risk among young women would have value in developing effective screening and prevention strategies at early ages. We have proposed that DNA methylation analysis of breast milk may provide breast cancer risk information among young women, and could possibly provide etiologic clues related to the higher rates of early onset cancers among African American as compared with White women in the US.

Objective: The purpose of this project was to identify associations between genome-wide DNA methylation levels in breast milk and race adjusted for other breast cancer risk factors.

Study Population: Cancer-free, lactating U.S. Black (n=57) and White (n=82) women, ages 19 to 44, provided frozen breast milk samples, as well as demographic, behavioral, and reproductive data, to the Breastmilk Laboratory at University of Massachusetts Amherst. Women were uniparous and did not have a personal history of breast cancer at the time of milk donation.

Methods: DNA was extracted from breast milk samples using the phenol-chloroform method. Genome-wide methylation analysis was performed on breast milk samples using the Infinium HumanMethylation450 BeadChip. Probes with 50% or more missing data, cross-reactive probes, as well as probes with minor allelic frequency greater than 0.05 in European- or African-Americans were removed, leaving 379,042 CpG sites for analysis. Multivariate generalized linear regression models were used to examine associations between race and other breast cancer risk factors and methylation beta values, adjusting for potential confounding factors. P-values less than 1E-7 were considered statistically significant.

Results: Black women in this study were more likely to be never smokers, to not have used over-the-counter pain medication in the past week, and to breastfeed longer. After adjustment by age, BMI, smoking status, and batch number, race was significantly associated with differential methylation at 1143 CpG sites, including 1024 at which Black women demonstrated increased methylation levels. Additionally, breastfeeding duration was associated with 269 CpG sites, with 268 showing a significant inverse relationship with methylation. Methylation sites significantly associated with Black race and lactation duration were located within tumor suppressor and promoter genes as well as in genes implicated in obesity and diabetes.

Conclusion: This preliminary analysis of DNA methylation in breast milk suggests that Black women have increased methylation and longer breastfeeding is associated with reduced methylation. Further research to understand how etiologic factors related to breast cancer may alter DNA methylation patterns in normal breast may lead to improved understanding of breast cancer risk at a young age and potentially causes of racial disparities in breast cancer incidence between White and Black women.
Atypical ductal hyperplasia and rate of upgrade to carcinoma on excision at a safety net hospital

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Purpose: Atypical ductal hyperplasia (ADH) is a high-risk lesion associated with an increased risk of developing breast cancer. Surgical excision following diagnosis of ADH on core needle biopsy is generally recommended due to high rate (10-30%) of upgrade to malignancy. Studies have been done to identify factors which may allow for observation rather than excision for management of ADH, however these have not been done in patients who obtain care from safety net health systems. The objective of our study was to examine the rate of upgrade to malignancy in a safety net hospital and to describe factors that may be associated with upstage.

Methods: A retrospective review identified women diagnosed with ADH on core needle biopsy from 2002-2015. Women with a concurrent diagnosis of ductal carcinoma in situ (DCIS) or invasive cancer were excluded. Only women who underwent excision were included in the comparative analysis. Upgrade (UG) was defined as histopathologic diagnosis of DCIS or invasive carcinoma on the excised specimen. Univariate analysis was used to compare baseline and clinical characteristics of the UG vs. non-upgraded (Non-UG) group. Logistic regression was performed to identify factors independently associated with increased odds of upgrade.

Results: ADH was diagnosed in 157 women and 122 (78%) underwent excision. The mean age was 53.3 ± 9.3 years, 48% were Hispanic, and the most common BIRADS score was 4A (58%). On diagnostic imaging, 78% had calcifications, 30% had a mass, and 6% architectural distortion. Time to excision from biopsy varied (median 2.3 months), however most women (81%) underwent excision within 6 months. 35 (29%) patients were upstaged to cancer (22 DCIS, 13 invasive cancer). UG was associated with African-American race (54% vs. non-UG 23%, \( p < 0.01 \)), history of non-breast cancer (11% vs. non-UG 0, \( p = 0.01 \)), BIRADS 5 (9% vs. non-UG 0, \( p = 0.02 \)) and mass on physical exam (36% vs. non-UG 15%, \( p = 0.03 \)) or diagnostic imaging (45% vs. non-UG 25%, \( p = 0.03 \)). Median follow up for the entire cohort was 813 days (IQR 327-1492). In Non-UG, 2 women subsequently developed cancer in a different area from the ADH. On logistic regression, African-American women (compared to Hispanic women, odds ratio [OR] 5.5, 95% confidence interval [CI]: 1.5-20.0, \( p = 0.01 \)) and those with a higher BI-RADS score of 4C and 5 (OR 13.2, 95% CI: 1.6-110.6, \( p = 0.02 \)) had increased odds of upstaging to malignancy.

Conclusion: The rate of upgrade from ADH to cancer was 29% in our cohort. Women diagnosed with ADH in safety net hospitals, particularly African-American women and those with high BI-RADS score (4C and 5), should be strongly advised to undergo excision. Further study on the impact of race on the rate of ADH lesions upgrading to malignancy on surgical excision is warranted.
Body: Aromatase inhibitors (AIs) have been used in the adjuvant treatment of postmenopausal women with hormone receptor positive early breast cancer as a consequence of the significant benefit in DFS and OS when compared with tamoxifen. However the patients who receive AIs have an increased risk of arthralgia, at most 50% of patients did not take AIs and the 20% of the discontinued patients were within the first year of use. The HOPE study demonstrated that exercise was effective in improving AI-induced arthralgia. We conducted the AIAI (Arthralgia Improvement for the patients with Aromatase Inhibitors) study using wider eligibility criteria that the HOPE study to assess the impact on AI induced arthralgia in breast cancer patients. Patients were randomly assigned, in a 3:1 ratio, to exercise intervention or usual care. Following randomization participants could choose from 3 types of exercise including group 1 (120-150 minutes per week of walking or running), group 2 (daily NIPPON HOSO KYOKAI: NHK broadcast exercise in Japan) and group 3 (going up the stairs- frequency, etc). The primary endpoint was the arthralgia change at 6 and 12 months, which was assessed using the BPI (Brief Pain Inventory). Secondary endpoints included the BPI according to the completion rate of exercise (70% and more or less than 70%), the BPI change of the patients with arthralgia (the patients who had arthralgia at the time they enrolled this study; BPI worst pain $3\leq$), the BPI of the each exercise group, the BPI according to the duration of AIs therapy (24 months and more or less than 24 months), the correlation between the BMI change and the BPI change, adherence of AIs and safety.

102 were randomly assigned to exercise intervention group (22 patients dropped out of this study) and 37 to usual care group (9 patients dropped out of this study). Trends for differentiations of pain interference at 12 months was detected between exercise intervention group and usual care group, but the differences did not reach statistical significance ($p=.067$). There was statistically better pain interference of the 70% and more exercise completion group than the usual care group at 12 months (-0.29±1.22 for exercise intervention group and 0.33±0.88 for usual care group, $p=.002$). The change of pain interference was statistically better for the exercise intervention group than the usual care group at 12 months ($p=.017$, -0.61±0.69 for exercise intervention group and 1.14±1.56 for usual care group). There was statistically significant difference of pain interference between group 1 exercise intervention group and the usual care group at 12 months (-0.14±0.68 for group 1 exercise intervention group and 0.33±0.88 for the usual care group, $p=.009$). Tendencies were detected in the AIs therapy less than 24 months group. Trends for the correlation between BPI and BMI were detected in worst pain at 6 month, pain severity at 6 month and pain interference at 12 month. There was a statistically significant difference of AIs adherence between the exercise intervention group (99%) and the usual care group (92%) ($P=0.03$).

Exercise may be effective in improving and preventing AI-induced arthralgia.
Title: Voice of cancer patients: Analysis of patient concerns regarding scalp cooling devices for prevention of chemotherapy induced alopecia

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Body: Adjuvant chemotherapies for breast cancer cause hair loss in majority of patients. Although Chemo Induced Alopecia (CIA) is temporary, is distressing for many patients and about 7% patients treated with docetaxel experience permanent hair loss. Scalp cooling devices (SCDs) have been shown to reduce hair loss in patients receiving chemo. Many patients share their experiences in online forums as freely shared messages and can be used to analyze concerns regarding CIA and SCDs. We use our automated system, VoCP, to analyze these messages and assess the utilization and efficacy of SCDs, concerns and associated barriers.

Method: We collected 15.13 million unique messages (987,189 patients) from 37 cancer forums that provide clinically relevant information; we built custom ontologies for chemo drugs and regimens, hair loss, SCDs, supportive therapy and efficacy. We then used VoCP (using techniques from Artificial Intelligence - deep learning, information retrieval and natural language processing) to extract relevant information.

Results: Out of 15.13 million, 17,966 messages from 4,258 patients discussed the use of SCDs during chemo. 16,527 of these were inquiring about SCDs, e.g., do SCDs work, side effects, Difficulty in use, risk of scalp and brain metastasis, cost and insurance coverage, ease of obtaining and manufacturers.

2,213 messages from 817 patients discussed SCDs with Docetaxel, 1,470 from 687 patients discussed with FEC/AC, and 517 from 249 patients discussed with AC/Taxol.

In 1,439 messages, 668 distinct patients who used SCDs mentioned that, they were able to keep 50-75% of their hair but experienced significant hair-thinning. Most users were satisfied with SCDs' use and would prefer them over losing hair completely; comments regarding side effects, barriers and advice included:

- Headaches, shivering, frost-bite, heaviness, neck and shoulder discomfort, chills, brain freeze, scalp pain, migraine, drowsiness, dizziness and cold; May use Lorazepam, Ibuprofen and Acetaminophen.
- Use mittens/hand-gloves, scarves, blankets and heating pads to keep body warm and avoid shivering
- Use cold caps during and 3-5 hours after chemo; change them every 15-30 minutes
- Cold caps with varying temperatures - warmer in front and sides for ear and forehead protection – may help; trim long hair
- For cold caps, either the facility needs to provide a freezer or patients need to bring their own
- Penguin cold caps are most common (2,055 messages) but monthly rent is $500-$600
- Patients can buy Elastogel caps (918 messages) for about $100
- Alternatives include icepacks/wrap-around provided by companies such as ElastoGel
- For single cap cooling devices such as DigniCap (337 messages) and Paxman (434), facility has to provide the system
- Rapunzel Project (171 messages) can help with scalp cooling gear, freezers and counseling; 79 SCD users mentioned getting their help

Discussion: Despite significant discomfort, cost and inconvenience, many patients are satisfied with SCDs’ use for CIA. More programs like Rapunzel project are needed to help patients. By analyzing millions of messages, VoCP provides meaningful insights into patients’ treatment and concerns and gives insight into unmet needs for further research and resources.
Title: Association of systemic paclitaxel concentrations with severity and progression of paclitaxel-induced peripheral neuropathy

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Body: Background: Paclitaxel is commonly used in combination regimens in patients with early stage breast cancer, but its use is often limited by the development of severe paclitaxel-induced peripheral neuropathy (PIPN). Paclitaxel pharmacokinetics (PK), specifically the maximum paclitaxel concentration (Cmax) and amount of time the concentration remains above 0.05 µM (Tc>0.05), have been associated with occurrence of severe, clinician-documentated (NCI CTCAE) neuropathy. Patient-reported outcomes are more reliable, sensitive, and responsive than clinician-documentated neuropathy, particularly for subjective toxicities. The objective of this study was to confirm that paclitaxel PK predicts occurrence and progression of patient-reported PIPN.

Methods: This observational trial enrolled patients with early stage breast cancer receiving adjuvant/neoadjuvant weekly 1-hour paclitaxel infusions (80 mg/m² x 12 cycles) at the University of Michigan Comprehensive Cancer Center. Patients with existing neuropathy or previous neuropathic chemotherapy treatment were excluded. Paclitaxel concentration was measured via liquid chromatograph/mass spectrometry (LC/MS) in plasma samples collected at the end of (Cmax) and 16-24 hours after (Tc>0.05) first infusion. Patient-reported neuropathy was collected (EORTC CIPN20) at baseline and each treatment cycle. The 8-item sensory subscale of the CIPN20 (CIPN8, range 8-32) was estimated for each patient and cycle of treatment to calculate the change from baseline (ΔCIPN8, range 0-24). The analysis was conducted using two approaches. In the first analysis, the cohort was stratified into cases and controls by the median ΔCIPN8 and in the second, each patient was described by the rate of change of CIPN8 per treatment cycle. The association of Cmax and Tc>0.05 with case/control definition was tested using Wilcoxon rank sum and t-tests. Associations with rate of change of CIPN8 were tested using linear regression.

Results: 60 patients were enrolled in this observational clinical study. The mean age was 52.4 years, 93% of patients were Caucasian, and 5% had diabetes diagnosis. At baseline there was very little patient-reported neuropathy (mean baseline CIPN8=8.3). The median ΔCIPN8 was 4. Patients with ΔCIPN8>4 had greater Cmax than patients with ΔCIPN8≤4 (p=0.010). In the regression modeling, there was a significant correlation between patient's Tc>0.05 and the rate of increase in ΔCIPN8 (r=0.28, p=0.03).

Conclusions: Our preliminary findings suggest that a single PK sample collected at the end of the first cycle (Cmax) or 16-24 hours later (Tc>0.05) are indicative of a patient's risk of experiencing PIPN during paclitaxel treatment. Ongoing modeling that accounts for dosing decreases, delays, and discontinuations will further characterize the contribution of paclitaxel pharmacokinetics to PIPN development and enable identification of genetic and metabolomic biomarkers that predict which patients experienced more severe PIPN than would be anticipated based on their paclitaxel PK.
Title: Effects of a combined exercise plus diet intervention on cardiorespiratory fitness among Japanese women with breast cancer: A feasibility study

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Body: Background
Remarkable deterioration of physical fitness is a well-established adverse event associated with endocrine therapy among breast cancer patients. Especially, impairment in cardiorespiratory fitness has been often reported in previous studies, affecting patients’ health and survival. Peak oxygen uptake (VO₂peak) is the gold standard for assessing cardiorespiratory fitness and is inversely correlated with cardiovascular disease among women with breast cancer as well as ordinal people. A number of previous studies have reported that aerobic exercise improves the risk of cardiovascular disease. Moreover, a proper diet program positively influences VO₂peak. However, almost all studies have been conducted in the Western community and there are few studies forcing on Asian women who have lower BMI compared with Western ones. Therefore, we investigated the feasibility of a combined exercise and diet program among Japanese cancer patients undergoing endocrine therapy and the effect on VO₂peak.

Methods
Thirty-Two Japanese women with breast cancer undergoing endocrine therapy (age; 50±6 years, body weight; 126±22 lbs) were voluntarily assigned to either intervention group (n = 21) or control group (n = 11). The intervention group completed a 12-week combined exercise plus diet program, consisting of weekly aerobic training and maintaining a nutritionally well-balanced 1,200 kcal/d diet. The control group were instructed to continue with their usual activities. Anthropometric indices, VO₂peak and QoL were measured at baseline and after the 12-week program. VO₂peak was assessed using an Okura protocol (Okura. 1999).

Results
All of the 21 women completed the 12-week program. The VO₂peak increased from 26.7 to 30.4 mL/kg/min (1.57 to 1.62 L/min) in the intervention group, while it remained unchanged (26.6 to 26.7 mL/kg/min) in the control group. Significant improvements were observed in VO₂peak and QoL in the intervention group (P < .001), while they remained essentially unchanged among the control group. Mean weight loss was 8.7% of the initial weight in the intervention group (P < .001) and 0.1% in the control group. No adverse events were reported in the intervention group.

Conclusions
Our combined exercise plus diet program may contribute to improvement in cardiorespiratory fitness, QoL, and body weight compared with control group. Importantly, cardiorespiratory fitness has been improved by as much as 15 % after three months. Further studies are needed to consider that what kind of aerobic exercise is best to improve cardiorespiratory fitness among Asian breast cancer patients.
Title: NEPA for CINV prevention in highly or moderately emetogenic chemotherapy – interim results of a German non-interventional study on quality of life and efficacy

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Body: Background
Inadequately controlled chemotherapy-induced nausea and vomiting (CINV) has a significant impact on the quality of life and daily functioning of cancer patients. Despite international antiemetic guidelines (ASCO, NCCN, MASCC/ESMO), CINV remains a problem for patients, especially in the delayed phase after chemotherapy application. International guidelines recommend a triple combination of 5-HT₃- and NK₁-receptor antagonist (RA) and dexamethasone given on day 1 for patients receiving HEC including anthracycline / cyclophosphamide (AC)-containing chemotherapy. The MASCC/ESMO guidelines recommend the triple regimen on day 1 also for patients receiving carboplatin-based MEC.

NEPA, a fixed dose combination of the NK₁-RA netupitant and the 5-HT₃-RA palonosetron, has been approved for the prevention of acute and delayed chemotherapy-induced nausea and vomiting (CINV) in cancer patients receiving cisplatin-based highly emetogenic (HEC) or moderately emetogenic chemotherapy (MEC).

Objectives
The primary objective of this prospective, non-interventional study is the evaluation of quality of life (QoL) in adult cancer patients receiving NEPA for CINV prevention in MEC or HEC. Secondary endpoints are efficacy and safety of NEPA.

Methods
The study is planned to enroll 2,500 cancer patients receiving single or two day MEC or HEC in German oncology centers. NEPA is prescribed in accordance with the marketing authorization. QoL is recorded in FLIE questionnaires. Efficacy, determined as complete response (CR, no vomiting, no rescue medication), additional medication, and adverse events are recorded in patient diaries and e-CRF. Three consecutive chemotherapy cycles must be documented.

Results
At the cut-off date 31 May 2017, 1,959 patients had been included. 86.3% of patients were female, with a median age of 57 years. 94.1% of patients had an ECOG Performance Status of 0 or 1. 67.4% of patients had breast cancer. 47% of patients received adjuvant, 31.7% received neoadjuvant chemotherapy.

At the cut-off date 31.05.2017, efficacy, assessed by physicians on a 4 point scale, was rated very good or good for 1,656 (89.7%), 1,540 (90.0%) and 1,469 (91.8%) patients in cycle 1, 2 and 3, respectively. The overall efficacy assessments of physicians and patients were very similar with approximately 90 % of good or very good efficacy of NEPA. Quality of life data as analyzed by 24 March 2017: Less than 10% of patients experienced reduced quality of life, with 90.8%, 92.1% and 90.8% reporting no impact on daily life due to vomiting for HEC in cycle 1, 2 and 3 and 91.7% and 93.6% for MEC. Nausea was harder to control. 64.0%-65.2% of the patients receiving HEC reporting no impact on daily life by nausea and 61.1% - 65.0% of the patients receiving MEC.

Conclusions
NEPA was very effective in the CINV-prevention in patients receiving HEC or MEC, with more than 90% of patients reporting no impact on daily life by vomiting. The study is ongoing.
Title: Efficacy of scalp cooling in preventing chemotherapy-induced alopecia in breast cancer patients: A retrospective, comprehensive review of 330 cases of Brazil

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Body: Background: Chemotherapy-induced alopecia (CIA) is a distressing adverse effect of many chemotherapy (CT) agents. New strategies for prevention of CIA have been studied. Scalp cooling has been reported to prevent CIA. We conducted a retrospective study aimed to assess the efficacy of scalp cooling in preventing CIA among women receiving chemotherapy for breast cancer. Methods: Was included clinical data of breast cancer patients at the Oncoclinicas Group from July/2015 to March/2017. All patients were elected to use scalp cooling to prevent CIA. Cooling started 30 minutes before infusion and was maintained throughout the infusion of the treatment and extended for 90 minutes after infusion. Degree of hair loss was rated by nurse assessment using CTCAE v4.0 scale in grade zero (without alopecia), 1 (<50%) or 2 (>50%), digital photographs and clinical assessment. Assessments were made before each chemotherapy treatment and at a follow up visit between 3 weeks and 3 months after the completion of chemotherapy. Success was defined when there was G0 or G1 alopecia at the end of the treatment, and failure when finished with G2 alopecia and patient withdrawal due to alopecia. Results: 330 patients were included. 283 with localized breast cancer and 47 with metastatic disease. 188 patients (57.0%) completed all treatment with scalp cooling. 72 patients (21.8%) withdrew from cryotherapy for alopecia of any degree, 51 patients (15.4%) gave up cryotherapy for complaints unrelated to alopecia and 19 patients (5.8%) had their treatment interrupted due to external factors (progression of disease, change of CT protocol, among others). Among patients who completed chemotherapy (n=188), the degree of alopecia at the end was G0 = 27, G1 = 138, G2 = 23. Thus, the overall success rate with cryotherapy was 63.5%. CT protocols initiated with doxorubicin and cyclophosphamide, followed by taxanes, presented a success rate of 50%. The combination of docetaxel and cyclophosphamide showed success of 71.9%.

Scalp cooling: chemotherapy regimes and alopecia

<table>
<thead>
<tr>
<th>Chemotherapy (CT) regime</th>
<th>Completed CT G0</th>
<th>Completed CT G1</th>
<th>Completed CT G2</th>
<th>Abandoned SC G1</th>
<th>Abandoned SC G2</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>AC/taxanes</td>
<td>6</td>
<td>51</td>
<td>5</td>
<td>25</td>
<td>27</td>
</tr>
<tr>
<td>AC non sequential</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D alone or combi no AC</td>
<td>3</td>
<td>19</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>DC</td>
<td>6</td>
<td>40</td>
<td>11</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>EC at 2nd part of CT</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>EC at 1rst part of CT</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>P alone or combi no AC</td>
<td>6</td>
<td>13</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>No AC</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AG at 2nd part of CT</td>
<td>3</td>
<td>10</td>
<td>3</td>
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<tr>
<td>Total</td>
<td>27</td>
<td>138</td>
<td>23</td>
<td>34</td>
<td>38</td>
</tr>
</tbody>
</table>

A: doxorubicin; C: cyclophosphamide; D: docetaxel; E: epirubicin; P: paclitaxel; combi: combination

In addition to alopecia, headache and cold sensation were common reasons for cryotherapy withdrawal. Conclusions: Scalp
cooling appears to be effective in preventing CIA among breast cancer patients who underwent chemotherapy. Studies involving a psychological approach to the expectation and experience of alopecia with cryotherapy and better management of pain are necessary to increase adherence to treatment.
Title: Effectiveness and adverse events of DigniCap® scalp cooling system

Lena Traub¹, Christiane Brandi¹, Fariba Khandan¹ and Marc Thill¹. ¹Agaplesion Markus Hospital, Frankfurt am Main, Hessen, Germany.

Body: Background
Chemotherapy-induced alopecia (CIA) is able to affect a patient's self-image and confidence negatively. A decade ago scalp cooling failed to avoid chemotherapy-induced hair loss in women who underwent chemotherapy as only thermal packs were used.

The DigniCap® scalp cooling system consists of a silicon cap that includes two sensor controlled cooling cycles. These cycles regulate the scalp temperature to lead to a continuous vasoconstriction in the scalp. Thus, it is indicated to reduce the likelihood of CIA.

Reduced temperature results in a reduced blood flow to the scalp area so that less chemotherapy reaches the hair cells. Therefore, these are not exposed to the full dose of chemotherapy and may be able to survive the treatment.

The purpose of this study was to evaluate the effectiveness and adverse events of DigniCap® scalp cooling system.

Patients and Methods
Since October 2015, 48 of 60 planned breast cancer patients undergoing chemotherapy were prospectively included in a unicentric cohort study in the certified breast cancer center at AGAPLESION Markus Hospital, Frankfurt, Germany. The average age of the women was 52.7 years (range 33 – 76).

The chemotherapy regimen included 4x EC q3w -> 12x paclitaxel q1w (60.4%), 4x TC q3w (8.3%), 6x carboplatin plus paclitaxel q3w (8.3%), 6x docetaxel, carboplatin, trastuzumab (TCbH) q3w + pertuzumab (4.1%), 4x nab-paclitaxel m 3 q4 (2.1%), 4x paclitaxel q2w -> 4x EC q2w (2.1%), 18x paclitaxel q1w (2.1%), 18x paclitaxel q1w plus myocet q1w (2.1%), eribulin d1, 8 q3 (2.1%), 4x EC q3w -> 12x paclitaxel q1w plus trastuzumab plus pertuzumab (2.1%), 6x carboplatin plus paclitaxel q3w plus bevacizumab (2.1%), 4x nab-paclitaxel3 q4 plus bevacizumab (2.1%), 4x nab-paclitaxel 3 q4 -> 4x EC q3w (2.1%).

Our aim was to quantify the grade of alopecia, satisfaction and side effects of the scalp cooling system. Alopecia quantification was done by a standardized questionnaire and photo documentation. Final results of the whole cohort of 60 patients will be presented at SABCS 2017.

Results
The interim analysis showed a success rate of complete hair loss avoidance of 8.0%. Hair loss of less than 20% was documented in 60.0% (29 patients). In 19/ 48 patients (39.6%) adverse reactions caused by the DigniCap® Scalp Cooling System, like headache (12.5%) or CIA (27.1%) were reported.

Conclusion
Our evaluation shows that DigniCap® Scalp Cooling System has a minimal rate of adverse events (39.6%) and reduces the likelihood of chemotherapy-induced alopecia (< 20% hair loss) effectively by 60.0%, even in anthracycline-based regimen.
Title: Chemotherapy-induced peripheral neuropathy and quality of life among breast cancer survivors

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Body: Introduction: Breast cancer survivors are at risk for long-term chemotherapy-induced peripheral neuropathy (PN), which has few treatment options and can affect quality of life. We examined 1) the presence and severity of PN and its correlates among 442 metastatic (MBC) and 238 breast cancer (BC) survivors who received chemotherapy, and 2) provider communication, preparation, and confidence in managing treatment side effects (SEs). Methods: MBC and BC survivors enrolled in the Cancer Support Community’s Cancer Experience Registry reported history of chemotherapy-induced PN and how disruptive (0=not at all; 4=very much) PN was to daily life. Participants also reported cancer-related distress (CancerSupportSource®, a 25-item measure with a 4-item depression/anxiety risk subscale) and quality of life (QoL; PROMIS-29). We examined the association between PN history, PN disruption, distress, risk for depression/anxiety, and worse QoL (PROMIS subscales >1SD from norms) with multivariate linear and logistic regression, adjusting for metastatic disease and number of comorbidities. We also explored associations between PN disruption, provider communication, preparation, and confidence in managing SEs. Results: Mean age was 55y (SD=10); 89% non-Hispanic White; 43% had ≥2 comorbidities. More MBC patients (66%) experienced PN than BC (54%; chi²=8.9, p<.01). Severity of PN disruption did not differ by metastatic disease; 30% of all participants indicated PN was quite a bit or very disruptive to daily life. Experiencing PN was associated with greater distress (beta=6.6, p<.001) and increased likelihood of risk for depression/anxiety (OR=1.77, 95% CI=1.26-2.48). The odds for worse sleep disturbance, pain interference, physical function, social role function, depression, fatigue, and anxiety were greater among those experiencing PN than not (n=266; ORs: 6.95-1.90 respectively; ps<.05). After controlling for metastatic status and number of comorbidities in separate regression analyses, greater PN disruption was associated with greater distress and risk for depression/anxiety (semipartial rs=.12-.19, ps<.01) and poorer QoL (semipartial rs=.22-.34, ps<.05), except for sleep disturbance where the relationship was not linear. Many survivors wished they received more help managing short-term SEs (39%) and long-term SEs (55%). Nearly one-third (32%) noted their provider had not suggested ways to cope with SEs and 33% were not confident in their ability to cope with SEs. Greater PN disruption was associated with wanting more help managing short-term SEs (n=121; p<.05) and not being counseled on ways to cope with SEs by the health care team (p<.05). Conclusion: Chemotherapy-induced peripheral neuropathy is associated with poorer functional outcomes and symptom burden among breast cancer survivors, whether or not their breast cancer has metastasized. Many survivors do not feel confident in coping with side effects and want more support in side effect management, yet a substantial proportion note their provider did not discuss coping strategies with them. Without effective pharmacologic intervention for PN, providers are encouraged to assess and refer survivors to appropriate supportive services, and PN should be considered as a meaningful endpoint in clinical studies.
Body: Background: In Mexico, approximately 30% of young women with breast cancer (YWBC) are childless and >40% express concern about infertility risk secondary to cytotoxic treatment. However, only 30% of patients recall being disclosed by their physician of such risk. The aim of this study was to characterize and analyze the caregivers' behavior, attitudes and knowledge towards fertility preservation in YWBC in a limited resource setting, such as Mexico.

Materials and Methods: A 20-item survey was designed and validated by an expert panel, which was answered by participants of the annual meeting of the Mexican Society of Oncology 2016, as well as by physicians affiliated to the same association via web. Pearson chi-square tests were used to assess factors associated with the likelihood of disclosure of infertility risk, discussion about methods of fertility preservation and referral to a reproductive health specialist.

Results: The participants' demographic characteristics are displayed in Table 1 and are associated with the main areas of interest in Table 2.

<table>
<thead>
<tr>
<th>Characteristics</th>
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<td>Not fair</td>
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<td><strong>Knowledge safety GnRH analogues</strong></td>
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The caregivers’ most influential factor in all areas was their self-reported sense of responsibility on disclosing patients about infertility risk. Those physicians that inform patients about infertility risk are statistically more likely to discuss fertility preservation strategies and to refer to a reproductive health specialist. As for the main barriers for fertility preservation, costs were the most frequently mentioned (29.6%), followed by lack of specialists (11.2%), and patient's prognosis according to clinical stage (11.2%).

**Conclusions:** This represents the first Latinamerican study evaluating the YWBC's caregivers' attitudes and practices towards fertility preservation, as well as their general knowledge concerning oncofertility issues. The fact that only one third of the enquired physicians were aware of the safety of ovulation inducers and use of GnRH analogues in YWBC is striking, which may translate into worse survivorship care. Furthermore, physicians reported that access barriers were the most prevalent factors that hindered appropriate referral. Health-care providers play a major role in the timely detection of the patient's interest in future offspring, thus it is crucial to promote knowledge about this relevant topic and endorse policies that can provide universal access to assisted reproductive technologies.
**Title:** Impact of disease progression on health-related quality of life in patients with metastatic breast cancer in the PRAEGNANT breast cancer registry

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**Body:** Background: Improved progression-free survival is considered as treatment goal for patients with metastatic breast cancer (MBC) since it is assumed to delay or prevent deterioration of quality of life. Aim of our analysis was to examine the influence of disease progression on health-related quality of life (HRQoL).

Methods: The PRAEGNANT study comprises a real-life registry for patients with MBC. HRQoL was assessed with the EORTC-QLQ-C30 Version 3.0 questionnaire at study entry and every 3 months thereafter. The primary endpoint was minimally important deterioration (MID) in global HRQoL score by ≥ five points between baseline and any follow-up assessment. A logistic regression model was built with MID (yes/no) at a follow-up timepoint as outcome variable and several covariates as predictors.

Results: In total, 329 patients were included in this analysis, with disease progression in 63 patients. Concerning the primary study aim, progression status predicted MID of global HRQoL status in addition to the other covariates. The adjusted odds ratio for the effect of progression status on MID was 2.22 (95% CI: 1.04 – 4.73). Comparisons of mean differences of QoL domains/scales yielded no differences.

Conclusions: We provide evidence that disease progression in patients with metastatic breast cancer in a real-world registry has a significant negative impact on HRQoL as measured by MID of HRQoL. This study emphasizes the relevance of avoiding progression and prolonging PFS to maintain QoL.
Title: Single-dose fosaprepitant for the prevention of chemotherapy-induced nausea and vomiting in patients with breast cancer receiving moderately emetogenic chemotherapy regimens

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Body: Background: Aprepitant has demonstrated efficacy in patients with breast cancer receiving anthracycline and cyclophosphamide (AC)-based chemotherapy, but the efficacy of single-day fosaprepitant in patients receiving moderately emetogenic chemotherapy (MEC) regimens not including AC has not been widely reported. In a post hoc analysis, we explored prevention of chemotherapy-induced nausea and vomiting (CINV) in a subgroup of patients with breast cancer using a single-day triple-antiemetic fosaprepitant (FA) regimen compared to a standard 3-day control regimen.

Methods: This was a global, randomized, double-blind, parallel-group, phase 3 study in adult subjects who were scheduled to receive an intravenous (IV) dose of ≥1 MEC on the first day of treatment (NCT01594749). AC regimens were excluded as they are no longer considered MEC. Subjects were randomly assigned to a control or FA regimen (1:1). The control regimen consisted of oral ondansetron 8 mg, dexamethasone 20 mg, and IV saline as placebo before the first dose of MEC on day 1, and oral ondansetron 8 mg 8 hours after the first dose and every 12 hours on days 2 and 3. The FA regimen consisted of the same dose of oral ondansetron on day 1, along with dexamethasone 12 mg and a single dose of IV FA 150 mg before the first dose of MEC on day 1, with no additional prophylactic antiemetic beyond day 1. The primary end point was complete response (CR; no vomiting or rescue medication) in the delayed phase (25-120 hours after chemotherapy initiation).

Results: Overall, 1000 subjects were included in the intention-to-treat population (FA: N = 502; control: N = 498), and the primary end point was met (P < 0.001; FA vs control). In a subset of 231 subjects with breast cancer, 110 received the FA regimen and 121 the control regimen. Subjects were female, with the exception of 1 male in the FA group, most were aged 50 years or older (67%), and 210 subjects (91%) received single-day MEC regimens on day 1. Among them, 17 subjects in the FA group (8.1%) and 27 in the control group (12.9%) received carboplatin-based chemotherapy, and the remaining 166 subjects received other MEC regimens. CR in the delayed phase was achieved by 76.4% of subjects in the FA group and 68.6% in the control group (difference, 7.8%). Approximately 79% in each group had no vomiting episodes in the overall phase (hours 0-120). Completion on study medication was high, at approximately 98% in each group. Adverse events (AEs) were similar between groups: overall AEs occurred in 79.1% and 73.6% of subjects in the FA and control groups, respectively. AEs considered treatment related by the investigator were also balanced by treatment arm, occurring in 14 (12.7%) and 13 (10.7%) subjects in the FA and control groups, respectively; there was 1 treatment-related serious AE (hypersensitivity) that occurred in the FA group.

Conclusions: A single-day IV FA regimen is effective for preventing CINV in breast cancer patients receiving MEC.
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Publication Number: P6-11-12

Title: Health-related quality of life, symptom burden and management in older women with breast cancer across the illness trajectory

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Body: Background: Symptom burden has a negative impact on Health-Related Quality of Life (HRQoL). Identifying symptom burden and sources utilized for symptom management at different points in the cancer trajectory may guide development of supportive care services for older patients with breast cancer.

Methods: This was secondary analysis of survey data from the University of Alabama at Birmingham Health System Cancer Community Network, which includes 12 hospitals across the Southeast. Female Medicare beneficiaries with breast cancer were surveyed from 2013-2015 via telephone. Demographic information, treatment status, HRQoL (Short Form Health Survey, SF-12), symptom burden (MD Anderson Symptom Inventory, MDASI), and sources used for symptom management (e.g. doctors, nurses) were collected. Descriptive statistics were computed for all variables of interest. HRQoL, overall symptom burden, and individual symptom scores by treatment status were compared using standardized mean differences (Cohen's d) and Student's t-tests. Simple linear regression was used to determine the association between symptom burden and HRQoL scores. A 10% False-Discovery-Rate was used to adjust for multiple inference.

Results: 321 women age 65 years and older with breast cancer were included in the survey sample; 141 (45%) were on treatment with chemotherapy (9%), radiation (1%), recurrence treatment (6%), or other planned cancer treatment (29%). Similar HRQoL scores were observed for participants on and off treatment, with SF-12 physical scores of 39.2 (SD 11.5) vs. 41.4 (SD 11.9, d=.19) and SF-12 mental scores of 52.5 (SD 9.7) vs. 53.2 (SD 9.4, d=.07). Symptom burden was also similar, with overall MDASI severity scores of 2.5 (SD 1.9) and 2.2 (SD 1.7, d=.17). For participants on treatment, fatigue and disturbed sleep were the most severe symptoms with average scores of 4.4 (SD 3.0) and 3.6 (SD 3.2), respectively. For participants off treatment, the most severe symptoms were fatigue and memory problems with average scores of 3.8 (SD 3.1) and 3.1 (SD 2.7). A small difference in disturbed sleep was observed, with participants on treatment reporting more symptom severity (3.6 [SD 3.2] vs 2.7 [SD 2.9], d=.3, p=.01). Minor differences were observed for the severity of other specific symptoms between the groups. For patients both on and off treatment, MDASI scores were strongly and significantly associated with decreased SF-12 physical (on: β=-3.0 [SE 0.4], R²=.21, p<.001; off: β=-3.8 [SE 0.4], R²=.34, p<.001) and SF-12 mental scores (on: β=-3.1 [SE 0.3], R²=.34, p<.001; off: β=-3.1 [SE 0.3], R²=.34, p<.001). The three most common sources to manage symptoms used by participants in both groups were nurses (47%), doctors (26%), and friends/neighbors (26%).

Conclusion: Quality of life and overall symptom burden were similar for patients on and off treatment, with only a small difference in disturbed sleep. Overall symptom burden was strongly associated with decreased HRQoL among older females with breast cancer. Nurses were the most common source utilized for symptom management, suggesting the importance of systematic trainings, particularly for nurses, on symptom management and supportive care services for patients on and beyond treatment.
Title: Efficacy of procyanidin rich therapeutic agent on surgical wound healing in breast cancer patients: A prospective, randomized, single blinded, single-center study

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Body: Purpose:
The purpose of this study is to evaluate the efficacy of dressing material containing plant extracts which is rich in procyanidins, Neo dermal activator (NDA), on surgical wound of breast cancer patients.

Methods:
This prospective randomized, controlled study assessed 54 patients who underwent breast cancer surgery. Patients were randomly divided into two groups before definitive surgery in the operating room: 27 patients using NDA and 27 patients using conventional wound care. We applied conventional wound dressing immediately after surgery to the control group and added Neo dermal activator to NDA group. Surgical site infection (SSI) rates within POD 7 were evaluated. After 6 months from surgery, we evaluated wound condition and patient satisfaction using visual analogue scale score.

Results:
SSI rates within POD 7 between two groups were not significantly different. One case of SSI was reported during 1 month after surgery in both groups, respectively. Satisfaction for sense was not different between two groups. Meanwhile, satisfaction for appearance ($p=0.011$), scar ($p=0.046$), and self-confidence in wearing exposed clothes ($p=0.019$) showed higher scores in NDA group. Total score of patient satisfaction was higher in NDA group ($p=0.019$). Spearmann correlation showed strong correlation between scar and self-confidence in wearing exposed clothes. ($\rho=0.773$, $p<0.001$)

Conclusion:
The use of procyanidin rich therapeutic agent may be safe and increase quality of life in breast cancer patients, especially in scar satisfaction and self-confidence in wearing exposed clothes. In addition, satisfaction for scar might affect emotional part of breast cancer survivors.
Title: Safety and effectiveness of sensor-controlled scalp cooling to prevent alopecia in primary breast cancer patients receiving neoadjuvant or adjuvant epirubicin, taxanes, or both

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Body: Background: Chemotherapy (Ctx)-induced alopecia (CIA), produces a deep psychological distress in many women involved. Sensor-controlled scalp cooling (SCSC) is now approved by the FDA due to both its effectiveness and safety to prevent CIA demonstrated in two randomized trials in patients (pts) with primary breast cancer (PBC). However, SCSC is still infrequently used in many countries due to physicians’ concerns regarding both its safety and feasibility, in particular regarding anthracycline-based Ctx [Nangia et al., 2017]. This retrospective analysis was initiated to obtain more detailed information on the effectiveness and safety of SCSC using the Paxman system (Paxman, Huddersfield, UK) in PBC pts exposed to CIA-inducing neoadjuvant (NACT) or adjuvant Ctx (ACT) based on epirubicin (E), taxanes (T), or both (ET) in the clinical routine. Methods: 79 pts who underwent SCSC alongside with Ctx for PBC from 2014-2017 were identified from our database: NACT, 41 (51.9%); ACT, 38 (48.1%); dose-dense (dd) Ctx, 56 (70.9%); non-dd Ctx 23 (29.1%); premenopausal, 44 (55.7%); postmenopausal, 35 (44.3%). The following Ctx regimens were used: E, 1 (1.3%); T, 23 (29.1%); ET, 55 (69.6%). Pts were subjected to SCSC during each Ctx cycle. CIA was quantified according to the Dean score (DS) determined 3 wks after the last Ctx cycle. Data were analyzed regarding feasibility indicated by the SCSC completion rate, quality of hair preservation (success: DS 0-2, failure: DS 3-4), reasons of SCSC discontinuation, and safety. Moreover, the following parameters were investigated in regard to the success of SCSC: menopausal status (pre- vs postpenopausal), NACT vs ACT, dd Ctx vs non-dd Ctx, E- or ET-based Ctx vs T-based Ctx. Results: 55 pts (69.6%) completed SCSC, with 36 (45.6%) experiencing complete hair preservation (DS 0), and 19 (22.8%) showing partial success (DS 1-2). 24 pts (30.4%) discontinued SCSC, with CIA being the main reason in 18 pts (22.8%). Headache or earache was reported in 2 pts (1.3%) each, and local discomfort in another 4 pts (5.1%). Side effects were all not severe and resolved quickly after cessation of SCSC. SCSC was equally effective in most analyzed subgroups. The relative risk (RR) to experience CIA was 1.11 (CI: 0.82-1.54) for post- vs premenopausal pts, 1.11 (CI: 0.83-1.53) for ACT vs NACT, and 0.99 (CI: 0.72-1.43) for dd Ctx vs non-dd Ctx. Pts receiving E or ET had a significantly higher RR for SCSC failure: 1.39 (CI: 1.04-1.81, p=0.035). However, the success rate in this group was still 62.5%, thus clearly indicating a clinically meaningful benefit. Conclusions: In our study, SCSC was feasible, safe and effective in order to prevent CIA in pts with PBC. All therapeutic subgroups benefited from SCSC. Although patients on E or ET are at higher risk for CIA, the success rate in this subgroup indicates that SCSC can be reasonably offered to patients subjected to NACT or ACT with epirubicin-containing regimens.
Body: Background: In México, there are more than 57 million women with breast cancer, and more than 7 million of these patients live in Mexico State; 12.4% of breast cancer diagnosis are being made in here. The diagnosis and treatment of patients with breast cancer changes their quality of life (QOL) on a physical, psychological, social and sexual basis.

Objectives: The main purpose of this study is to examine the changes in the QOL of breast cancer patients treated at the State of Mexico Cancer Center (ISSEMyM), applying the EORT QLQ-C30 and QLQ-BR23. The secondary aim of the study is to describe the Health-Related Quality of Life (HRQoL), including partner relationships, sexual function, and body image concerns of breast cancer survivors. (BCS).

Design: A longitudinal, prospective, descriptive and analytical cohort study was made; with bivariate analysis and correlations search. We used Kolmogorov-Smirnov test for normal continuous variables. To evaluate changes in follow-up we use ANOVA test of repeated measures and post-hoc analysis. Significance was set at p <0.05

Method: QOL questionnaires were applied every 4 months followed for a year, the first one being done before the patient was informed of confirmed breast cancer diagnosis. A sample of 112 women with primary breast cancer were enrolled. The sample type is probability, non-confessional, consecutive cases.

Results: Primary cancer treatment has a negative impact on QOL comparing to data collected at the basal assessment (before confirmatory diagnosis of cancer). Average age 54 years (25-85). FIGO stage in situ in 5%, Ia, Ila and Iib 18%, IIa 8%, IIb 6%, IIIc 12% and IV 15%. LuminalA 40%, LuminalB 24%, Her2 15%, Triple negative 21%. The mean overall health score was 64.7±26.7 at baseline and 65.8±19.3, 65.3±19.6, 72.1±15.1 at 4.8 and 12 months, respectively. Physical, emotional, cognitive, sexual and social functioning, as well as symptoms and sexual pleasure present statistical significance (p.0000) regardless of age, stage or treatment received.

Conclusions: This is the first longitudinal, prospective, descriptive and analytical cohort made in Mexico that assesses changes in QOL of breast cancer patients. There is a significant decrease in post-surgery physical function in our patients. However, there is no significant difference between the results observed in patients undergoing conservative surgery versus those who received radical surgery, nor for patients receiving surgery plus radiation therapy versus surgery plus systemic therapy. Also in the body image category component in our study, an important decrease in the QOL of the patients undergoing surgery is demonstrated, this is more evident in early stages of disease. In patients who recieved chemotherapy, symptoms were a negative influence in QOL and just near a half came to a basal status a year after treatment started. Finally, patients with surgery as first treatment have a faster return to their basal QOL.

This work opens an invaluable opportunity to improve our medical behavior towards breast cancer patients, treated at the ISSEMyM State of Mexico Cancer Center. It obliges us to create a multidisciplinary team that permanently assesses patients, identifying the aspects of their daily work, which alter their QOL.
Title: Dysgeusia, weight and eating habits changes in breast cancer patients undergoing chemotherapy: A prospective cohort study

Rebecca Pedersini¹, Alessandra Zanini¹, Michela Romelli², Veronica Coccoli², Mafalda Guerra², Sara Bosio², Gabriella Schivardi², Filippo Rodella¹, Lucia Vassalli¹, Vito Amoroso², Melanie Claps², Edda Lucia Simoncini¹ and Alfredo Berruti². ¹Spedali Civili, Brescia, Italy and ²Spedali Civili, Brescia, Italy.

Body: Background: Dysgeusia is a frequent side effect in breast cancer patients undergoing adjuvant chemotherapy. The effect of dysgeusia on changes in diet and patient weight has been poorly investigated. We evaluated prospectively dysgeusia in a consecutive series of early breast cancer patients (EBCP) with the aim to explore its impact on food selection and weight changes.

Patients and methods: From May 2014 to April 2017, 130 consecutive EBCP were enrolled at the Oncology and Breast Units at Spedali Civili in Brescia. Dysgeusia was categorized as present or absent, changes in types and frequencies of food consumptions during chemotherapy were assessed by a specific questionnaire and photography atlas. Evaluations were performed at baseline, after 3 cycles, at the end of chemotherapy plan and during follow-up.

Results: all 130 pts were evaluable. Median age was 53 years (range 26-76), median weight 64 Kg (range 45-115), median BMI 23.86 Kg/m² (range 17.01-35.49), 73 pts (56%) performed physical activity, 57 pts (44%) were abstemious, 22 pts (17%) received neoadjuvant and 108 pts (83%) adjuvant chemotherapy, 25 pts (19%) were treated with antracycline based schemes, 83 (64%) with antracycline and taxane plus or minus trastuzumab, and 22 pts (17%) other regimens. Seventy-three per cent of pts developed dysgeusia during chemotherapy (p=< 0.001), and in 19% this side effect persisted three months after chemotherapy. Dysgeusia was associated with the length of chemotherapy treatment and taxane use. A statistically significant increase of taste alteration was reported for all metallic, sweet, bitter, salted and acid taste (p<0.001). Dysgeusia was more frequent in pts reporting meteorism and fatigue and in pts without oral mucositis (p=0.002). Dysgeusia correlated with a lower intake of bread, cheese, fat salami, butter and wine and a high intake of biscuits and cakes. However, no correlation was observed with weight and BMI changes.

Conclusion: Dysgeusia was frequent in EBCP undergoing adjuvant chemotherapy. It directly correlated with fatigue and meteorism and inversely correlated with mucositis. This adverse event had no impact on weight and BMI but significantly influenced food intake with a preference of glucose containing instead of fat containing foods.

Acknowledgments: a thank you to Beretta foundation for the constant support to Breast Unit and Oncology Department.
Title: Nanoparticle albumin-bound paclitaxel-induced peripheral neuropathy in patients receiving neoadjuvant or adjuvant chemotherapy for breast cancer

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Body: A brief background discussion. Nanoparticle albumin-bound paclitaxel (nab-PTX) has been developed under the concept of improved drug delivery. nab-PTX has been shown to significantly increase progression-free survival compared with solvent-based paclitaxel (PTX) in metastatic breast cancer. However, the long-term outcomes of nab-PTX induced peripheral neuropathy (nPIPN) have not yet been fully elucidated.

Trial design: observational cohort study (UMIN20852)
Primary objective: The long-term outcome of nPIPN
Secondary objectives
1) The validity of the Neuropathic Pain Screening Questionnaire (Japan–Q, Ishikawa et al. Pain Research 2016)
2) To evaluate the effect of frozen gloves and elastic stockings for nPIPN

nPIPN was assessed by the Japan-Q (J-Q) and Common Toxicity Criteria for Adverse Events ver 4.0 (CTC). The J-Q is an assessment tool for patient-reported pain severity, which consists of seven items, each rated on a five-point scale 0-4; grade 0 (no symptoms) to grade 4 (very severe symptoms). Pricking pain, electric shock pain, burn like pain, tingling pain with numbness, allodynia, reduced or hyper sensitivity and hand-foot syndrome. Assessment was performed every day during chemotherapy, six months and one year after completion of nab-PTX therapy. Total of 28 points for each day and 588 points for each cycle, higher point correlates with severity. Frozen gloves during administration of nab-PTX and/or elastic stockings were optionally used.

Statistical methods
Kruskal Wallis test was used and p<0.05 was considered as significant.

RESULTS:
Between May 2013, and April 2016, 105 patients were enrolled from 7 hospitals. In 73 patients (69.5%) nab-PTX was administered as primary and in 32 patients (30.5%) as adjuvant therapy. Forty three (41.0%) patients received nab-PTX as first line and 62 (59.0%) received after anthracycline contained regimen. Trastuzumab was administered as combination therapy with nab-PTX for Her2 positive patients. Both frozen gloves and stockings were used in 21 patients (20.0%) and frozen gloves only were used in 21 patients (20.0%) according to patient preference.

One hundred patients (95.2%) completed four courses, and overall relative dose intensity was 91.4%.
During chemotherapy, J-Q scores go elevated from day 1 to day 5 and gradually declined throughout the rest of the cycle. Without using frozen gloves, there was a significant increase according to courses (1st: 45.0 ± 5.7, 2nd: 76.7 ± 8.6, 3rd: 94.3 ± 11.8, 4th: 95.4 ± 11.8). Using frozen gloves, there was a significant increase from 1st course to 2nd, but no further increase was observed (1st: 37.4 ± 8.1, 2nd: 61.9 ± 12.4, 3rd: 62.9 ± 10.4, 4th: 55.3 ± 10.6). After six month and one year, the scores were significantly lower compared with the last day of the fourth cycle (4.28 ± 0.50, 2.53 ± 0.25, 2.85 ± 0.39, respectively). CTC, grade 2 or more sensory disturbance was observed in 57.9% after four cycles, but improved to 9.5% and 5.4% after six month and one year respectively.

CONCLUSIONS:
Patient-reported nPIPN was significantly getting worse without frozen gloves during chemotherapy, however be largely reversible within 1 year of PST or adjuvant treatment. The J-Q findings support that nab-PTX treatment is tolerable.
Title: Ten year trends in antiemetic prescribing in cancer patients receiving highly emetogenic chemotherapy (HEC)

Ciara C O'Sullivan¹, Holly K Van Houten¹, Lindsey Sangaralingham¹, Alexis D Leal¹, Shivani Shinde¹, Hongfang Liu¹, David Ettinger², Charles L Loprinzi¹ and Kathryn J Ruddy¹. ¹Mayo Clinic, Rochester, MN and ²Johns Hopkins Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD.

Body: Purpose: Prevention of chemotherapy-induced nausea and vomiting (CINV) is essential to preserve quality of life in cancer patients receiving highly emetogenic chemotherapy (HEC) such as doxorubicin-cyclophosphamide (AC) or cisplatin. Recently, new drugs (e.g. fosaprepitant and newer neurokinin 1 receptor antagonists [NK1RAs], rolapitant and netupitant) and updated guidelines for antiemetic use (e.g. adding olanzapine) have emerged. However, trends in real world antiemetic use are understudied.

Methods: We performed a retrospective study using the OptumLabs Data Warehouse (OLDW), which includes administrative claims for privately insured and Medicare Advantage enrollees in the U.S. We identified 34,236 patients age 18 years or older treated with either AC or cisplatin between January 2006 and June 2016. Data collected included baseline demographics (age, gender, census region and race), chemotherapy administered, and presence/absence of a central intravenous access device. Trends of anti-emetic use were presented overall and separately for 5-HT receptor antagonists (5HT3RAs) and NK1RAs.

Results: 23,030 patients (67.3%) received an anthracycline-based regimen (AC with or without docetaxel or paclitaxel), and 11,206 (32.7%) patients received cisplatin. Approximately two thirds of patients were female (n= 23,392). Dexamethasone use was stable over the decade (used by 85-90% in all years). Use of 5HT3RAs, primarily palonosetron and ondansetron, occurred in at least 95% of patients in all study years, consistent with guideline recommendations. NK1RAs were underutilized early on compared with guideline recommendations, but use increased to approximately 80% in the most recently evaluated year. Fosaprepitant use rose precipitously starting in 2009, preceding a sharp fall in aprepitant use beginning in 2011. The use of olanzapine, rolapitant and netupitant was minimal throughout the study period.

Conclusions: Dexamethasone and 5-HT3RAs were used in the vast majority of patients receiving HEC, in accordance with guideline recommendations. Less compliance with guidelines was seen with NK1RA use.
Title: Efficacy and patient acceptability of the DigniCaP ScalpCooler to prevent hair loss in breast cancer patients receiving adjuvant chemotherapy

Lucia Vassalli¹, Rebecca Pedersini¹, Michela Romelli², Melanie Claps², Carla Fornaro², Elisa Conti², Mauro Tagliani², Arianna Baronchelli², Debora Ragni², Eleonora Lombardi², Filippo Rodella², Vito Amoroso², Alfredo Berruti² and Edda Lucia Simoncini¹. ¹Spedali Civili, Brescia, Italy and ²Spedali Civili, Brescia, Italy.

Body: Background: Alopecia is a common and distressing adverse effect in breast cancer (BC) patients (pts) receiving adjuvant chemotherapy. The aim of the study was to assess the effectiveness and safety of this device to prevent chemotherapy-induced alopecia in early breast cancer patients (EBCP) receiving adjuvant treatment. The quality of life of pts was also evaluated. Patients and methods: From January to December 2016, a sensor-controlled scalp cooling system (DigniCap: Sysmex Europe GmbH, Norderstedt, Germany) was proposed to a consecutive group of EBCP submitted to adjuvant chemotherapy at the Breast Unit of Spedali Civili Hospital of Brescia. Degree of hair loss was assessed by two nurse using Dean's alopecia scale by digital photographs at baseline and each chemotherapy cycle. EORTC QLQ-C30 questionnaire and self-reported visual analogical scale (VAS) of symptoms (anxiety, tone of mood, fatigue, nausea, well-being, activity) were collected at baseline and after the first two cycles of chemotherapy. Results: 70 pts were enrolled and 49 (70%) completed the chemotherapy plan and were evaluable. Median age was 51 years, 8 pts (16%) received neoadjuvant and 41 pts (84%) adjuvant chemotherapy, 21 (43%) were treated with 4 cycle of chemotherapy (TC, EC or paclitaxel alone), and 28 (57%) with sequential chemotherapy with anthracycline and taxane + trastuzumab. Fifteen pts (30%) stopped the treatment because of loss of hair in 9 pts, for headache in 4 pts and for other problems in 2 pts. At the end of chemotherapy, 13 pts (27%) had no loss of hair (Dean score 0), 25 pts (51%) had a minimal loss of hair (Dean score 1), 9 pts (18%) had a 50% hair loss (Dean score 2), 2 pts (4%) had a 75% hair loss (Dean score 3). No pts reported hair loss more than 75% (Dean score 4). There wasn't a significant difference between mean score value of QLQ-C30 at baseline and after chemotherapy and between the groups with and without hair loss. VAS documented an increase of fatigue and decrease of anxiety from baseline to final evaluation. The side effects presented with the use of DigniCap were the following: headache in 32% of pts and cold feeling in 57% of pts. Conclusion: Scalp cooling with cold caps appears to be effective in preventing CIA among the majority of women undergoing treatment chemotherapy. The quality of life did not change in scalp-cooled patients. Acknowledgments: a thank you to the ESA association that donated Dignicap to Oncology Department.
BODY: BACKGROUND: BREAST-Q has been designed to evaluate perception outcomes among women undergoing different types of breast surgery. Modules have been developed for patients undergoing mastectomy, breast reconstruction, augmentation, reduction/mastopexy and breast-conserving therapy.

OBJECTIVES: Generate a translated version that is conceptually equivalent to the original version and to validate for Mexican population.

METHODS: A linguistic validation was performed for the Mastectomy Module of Breast-Q which includes preoperative-test and postoperative-test. Reliability and validity tests were performed. Test-retest was carried out in selected patients.

RESULTS: A total of 6 Mexican Mestizo Women completed preoperative-test questionnaires and 15 women completed postoperative-test questionnaire. All patients were planned for mastectomy or a mastectomy was performed. Median age was 53.0 (range 40-76), clinical stages were Stage II 9 (42.8%) Stage III 12 (56.1%) and stage IV 0. Validity was supported by three Rasch analysis findings: Questionnaire compliance rates were high, and the instrument was well accepted; the internal consistency tests demonstrated good convergent and divergent validity.

<table>
<thead>
<tr>
<th>Subscale</th>
<th>No. of item</th>
<th>Chronbach’s $\alpha$</th>
<th>Subscale</th>
<th>No. of item</th>
<th>Chronbach’s $\alpha$</th>
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<tbody>
<tr>
<td>Satisfaction with Breast</td>
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<td>0.880</td>
<td>Satisfaction with Breast</td>
<td>4</td>
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<td>Physical Well-being</td>
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<td>16</td>
<td>0.738</td>
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<td>0.911</td>
<td>Sexual Well-being</td>
<td>6</td>
<td>0.974</td>
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</table>

N= 6 patients

With the exception of psychosocial well-being of Post-test Questionnaire, Cronbach’s $\alpha$ coefficients was acceptable.

<table>
<thead>
<tr>
<th>Subscale</th>
<th>No. of items</th>
<th>Chronbach’s $\alpha$</th>
<th>Subscale</th>
<th>No. of items</th>
<th>Chronbach’s $\alpha$</th>
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</thead>
<tbody>
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<td>Satisfaction with Breast</td>
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<td>0.770</td>
<td>Satisfaction with Breast</td>
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<td>0.753</td>
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<td>Psychosocial Well-being</td>
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<td>0.508</td>
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<tr>
<td>Physical Well-being</td>
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<td>0.946</td>
<td>Sexual Well-being</td>
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<tr>
<td>Satisfaction with Surgeon</td>
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<td>0.959</td>
<td>Satisfaction with Surgeon</td>
<td>12</td>
<td>0.950</td>
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<tr>
<td>Satisfaction with Medical Team</td>
<td>7</td>
<td>0.988</td>
<td>Satisfaction with Medical Team</td>
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<td>9.88</td>
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<tr>
<td>Satisfaction with Medical Team</td>
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<td>0.974</td>
<td>Satisfaction with Medical Team</td>
<td>7</td>
<td>9.85</td>
</tr>
</tbody>
</table>
N= 15 patients

Psychosocial Well-being Subscale Does not pass the test of reliability, after further analyze we must be restructure. Test-re-test reliability score ranged between >0.7 to 1.00.

CONCLUSIONS: The Mexican Spanish version of Mastectomy Module of Breast-Q is reliable and easy to implement in population with breast cancer in different scenarios in México with the advantage to measure quality of life and satisfaction in our population with locally advanced disease, this will be of help to improve quality of health care.
2017 San Antonio Breast Cancer Symposium

Publication Number: P6-12-01

Title: Can exercise influence survival following breast cancer? Evidence from randomised, controlled trials

Sandra C Hayes¹, Megan Steele¹, Rosa Spence¹, Louisa Gordon², Diana Battistutta¹, John Bashford³, Chris Pyke⁴, Christobel Saunders⁵ and Elizabeth Eakin⁶. ¹Queensland University of Technology, Brisbane, Queensland, Australia; ²QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia; ³Wesley Hospital, Brisbane, Queensland, Australia; ⁴Mater Public and Private Hospitals, Brisbane, Queensland, Australia; ⁵University of Western Australia, Perth, Western Australia, Australia and ⁶University of Queensland, Herston, Queensland, Australia.

Body: Background: The Exercise for Health (EfH) trials were randomized, controlled trials designed to evaluate an 8-month pragmatic, exercise intervention, commencing 6 weeks post-surgery for women with newly diagnosed breast cancer residing in urban- or rural/regional areas. Outcomes for these exploratory analyses were overall survival (OS), breast cancer-specific survival (BCS) and disease-free survival (DFS). Methods: Consenting urban-residing women (EfH 1, n=194) and rural/regional-residing women (EfH 2, n=143) were randomized to exercise or usual care. Cox proportional hazards models were used to estimate hazard ratios (HR) and 95% confidence intervals (CI) for OS, BCS and DFS (exercise group, n=207, 65% urban women; usual care group, n=130, 46% urban women), with and without adjustment for prognostic factors including trial (urban/rural), age, body mass index, disease stage and presence of comorbidities. Further exploratory subgroup analyses were also conducted to assess whether effect on OS, BCS and DFS differed according to prognostic variables. Results: After a median follow-up of 8.3 years (IQR: 8.0-8.7 years) there were 11 (5.3%) deaths in the exercise group compared with 15 (11.5%) deaths in the usual care group (Table 1).

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>Usual care</th>
<th>Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=337</td>
<td>n=130</td>
<td>n=207</td>
<td></td>
</tr>
<tr>
<td>Breast cancer-related deaths</td>
<td>20 (5.9)</td>
<td>10 (7.7)</td>
<td>10 (4.8)</td>
</tr>
<tr>
<td>Non-breast cancer-related deaths</td>
<td>6 (1.8)</td>
<td>5 (3.8)</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Overall survival events</td>
<td>26 (7.7)</td>
<td>15 (11.5)</td>
<td>11 (5.3)</td>
</tr>
<tr>
<td>Recurrence of breast cancer</td>
<td>20 (5.9)</td>
<td>8 (6.2)</td>
<td>12 (5.8)</td>
</tr>
<tr>
<td>Alive at follow-up</td>
<td>9 (2.7)</td>
<td>3 (2.3)</td>
<td>6 (4.6)</td>
</tr>
<tr>
<td>Deceased by follow-up</td>
<td>11 (3.3)</td>
<td>5 (3.1)</td>
<td>6 (4.6)</td>
</tr>
<tr>
<td>New primary breast cancer</td>
<td>13 (3.9)</td>
<td>5 (3.8)</td>
<td>8 (3.9)</td>
</tr>
<tr>
<td>Alive at follow-up</td>
<td>13 (3.9)</td>
<td>5 (3.8)</td>
<td>8 (6.2)</td>
</tr>
<tr>
<td>Deceased by follow-up</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Disease-free survival events*</td>
<td>48 (14.2)</td>
<td>23 (17.7)</td>
<td>25 (12.1)</td>
</tr>
</tbody>
</table>

* Disease-free survival events include recurrence of breast cancer, new primary breast cancers, and all-cause deaths. Note that women who had recurrence and died before follow-up only count once towards disease-free survival events.

HRs for the exercise group were: OS: 0.45, 95% CI=0.20-0.96; p=0.04; BCS: 0.61, 95% CI=0.25-1.46, p=0.26; and DFS: 0.66, 95% CI=0.38-1.17; p=0.16 (adjusted analyses yielded similar results). With the exception of BCS for those with a body mass index >30, all HRs for subgroup analyses favored exercise, with effect on OS for women of younger age (<55), those with stage II+ disease, and those with 1+ comorbidity at baseline significant (p<0.05). Effect of exercise on DFS was also significant (p<0.05) for urban women. Conclusion: Findings suggest that an individually-prescribed and monitored exercise program integrated during and beyond treatment for breast cancer, and that was designed to cater for all women, irrespective of place of residence and access to health services, has clear potential to influence survival.
2017 San Antonio Breast Cancer Symposium

Publication Number: P6-12-02

Title: Racial/ethnic differences in sleep quality and duration among breast cancer survivors: Results from the women's health initiative (WHI)

Chloe M Beverly¹ ², Michelle Naughton², Randi Foraker¹ ², Michael Pennell¹ ², Gregory Young², Lauren Hale³, Tracy Crane⁴, Kathy Pan⁵, Suzanne Danhauer⁶, Elizabeth Feliciano⁷ and Electra Paskett¹ ². ¹College of Public Health, The Ohio State University, Columbus, OH; ²Comprehensive Cancer Center, The Ohio State University, Columbus, OH; ³Stony Brook University School of Medicine, Stony Brook, NY; ⁴Arizona Cancer Center, The University of Arizona, Tucson, AZ; ⁵University of California, Los Angeles, Los Angeles, CA; ⁶Wake Forest University, Winston-Salem, NC and ⁷Kaiser Permanente Division of Research, Oakland, CA.

Body: BACKGROUND: Sleep is a crucial factor for optimal health, but breast cancer survivors often report poor sleep quality. It is estimated 20-70% of survivors have at least one sleep problem, which contribute to quality of life and health differences among survivors. Minority groups tend to have poorer sleep quality and shorter sleep duration than Non-Hispanic Whites (NHW). African-Americans (AA) with breast cancer have a poorer prognosis than NHW for each stage-specific diagnosis and are twice as likely as NHW to report short sleep duration, yet survivor studies are still lacking in AA participants. The purpose of this study was to examine sleep quality and duration patterns before and after cancer diagnosis by race/ethnicity among WHI breast cancer survivors.

METHODS: There were 12,098 postmenopausal women diagnosed with invasive breast cancer after WHI enrollment who were eligible for this secondary analysis. Baseline demographic and clinical characteristics were described. The WHI Insomnia Rating Scale (WHIIRS) was measured at multiple time points pre- and post-diagnosis. A higher WHIIRS scores (0-20 points) indicates greater sleep disturbance and ≥9 points identifies clinical insomnia. A linear mixed model was fit to the WHIIRS sleep quality data to examine if the trend in sleep quality with time changed following a cancer diagnosis. For short (<6hrs) and long (≥9hrs) sleep duration, we fit a logistic regression model with multilevel mixed effects.

RESULTS: The majority of participants were NHW (87.4%), mean age at diagnosis was 70.3 years, and 75% had localized breast cancer at diagnosis. At baseline, 30% of women had insomnia. The lowest average WHIIRS score was 5.6 among Asians, and the highest was 6.6 among American-Indians and NHWs (p=0.02). AAs had the most women sleeping ≤5 hrs/night and NHW had the least (19.6% vs 5.7%, p<0.01). At diagnosis, the average WHIIRS score was 7.2. After diagnosis, sleep quality improved in the overall study population (p=0.03). Short sleep duration ranged from 6% before diagnosis, 9% at diagnosis and 11% after diagnosis (p=0.29). Long sleep duration ranged from 3% before diagnosis, 6% at diagnosis and 15% after diagnosis (p=0.43). There was no difference in sleep quality across race after diagnosis (p=0.53). The probability of short sleep and long sleep after diagnosis did not differ significantly across race (p=0.12, p=0.90), however racial minorities tended to have higher probabilities of short sleep at diagnosis compared to NHWs.

DISCUSSION: Sleep is an appealing area to target for improvement due to the multiple ways it can be treated. With increasing survival rates, there is an emphasis on improving quality of life in survivors. Our results span 20 years pre-diagnosis to 15 years post-diagnosis and are similar to shorter follow-up studies which found most women's sleep problems resolve within a few years of treatment completion. The lack of difference by race was an unexpected finding in another similar longitudinal study, which suggested most differences are seen in cross-sectional sleep studies. This study adds to the literature on longitudinal sleep data, especially to the little data on sleep trajectories in minority breast cancer survivors.
Title: Utilization of bioimpedance spectroscopy in the prevention of chronic breast cancer related lymphedema

Body: Background: With improved breast cancer outcomes, an increasing focus on sequelae of treatment as part of survivorship has emerged. Breast cancer related lymphedema (BCRL) represents one such sequela. Increasing data and recent NCCN guidelines support the use of prospective BCRL surveillance to allow for early detection and intervention as a method to reduce chronic, irreversible BCRL. Therefore, this study was performed to evaluate the impact early detection and treatment of BCRL in breast cancer patients undergoing prospective surveillance with bioimpedance spectroscopy (BIS).

Methods: From 8/2010 through 12/2016, 206 patients were evaluated with BIS as part of a prospective surveillance program. The protocol included pre-operative assessment with BIS as well as post-operative assessments with BIS at regular intervals. Patients with L-Dex readings increasing by more than 10 from baseline were considered to have subclinical BCRL and treated with a compression sleeve for 4 weeks. For the purpose of this analysis, high-risk was defined as receipt of ALND, regional nodal irradiation, or taxane chemotherapy. Chronic BCRL was defined as the need for complex decongestive physiotherapy.

Results: A total of 206 patients were analyzed, with a mean age of 61 years old and a median follow up of 25.9 months. Overall, 17% of patients had at least one high-risk feature, 8% had two factors, and 7% had all three factors. A total of 21 patients (9.8%) were diagnosed with subclinical BCRL. Increased rates of subclinical BCRL were seen in patients undergoing ALND (23% vs. 7%, p=0.01) with ALND and receipt of RNI associated with development of subclinical BCRL. At last follow-up, no patients had persistent, chronic BCRL following early, conservative intervention measures.

Conclusions: The results of this study support prospective surveillance and early treatment utilizing BIS. Intervention triggered by subclinical BCRL detection with an elevated L-Dex score was associated with a very low rate of chronic BCRL.
Quality of life of Chinese breast cancer survivors in association with lifestyle changes before and after cancer diagnosis

Yuan-Yuan Lei¹, Iris Chi-Kiu Lee¹, Ka Li Cheung¹, Roselle Lee¹, Yiqian He¹, Winnie Yeo¹ and Suzanne C Ho¹. "Chinese University of Hong Kong.

Body: Background: Epidemiologic studies in the West have found that lifestyle factors, including maintaining normal body weight, being physically active and eating a healthy diet are individually associated with better quality of life (QOL) among breast cancer survivors. Limited data is available on lifestyle modifications in association with quality of life of breast cancer survivors in Asian region. The objectives of this study were to [1] determine the lifestyle changes among Chinese breast cancer survivors at diagnosis and 18-month post diagnosis; and [2] to assess the association of lifestyle changes with QOL.

Methods: In this prospective cohort study, 1300 Chinese breast cancer patients were assessed at breast cancer diagnosis (baseline; reflecting pre-diagnosis) and at 18-month post-diagnosis. During each assessment, individual patient's lifestyle within the previous 12 months were recorded and included exercise, diet, and body mass index (BMI) data; each patient also underwent self-administered QOL assessment. Assessment of lifestyle modifications were based on World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) recommendation adherence scores (range: 0-6).QOL was evaluated by European Organization for Research and Treatment of Cancer-Quality of Life Questionnaire C-30 (EORTC-QLQ-C30). Paired t-test was performed to compare the overall recommendation adherence score before and after diagnosis. To investigate the association between recommendation adherence score and HRQoL, generalized linear models were used to compare the least-square means by tertiles of adherence score (T1, T2, and T3) and continuous adherence score. In the multivariate models, adjustment were made for age, stage of cancer, education level, marital status, comorbidities, smoking status, current hormonal therapy and energy intake.

Results: The mean recommendation adherence score significantly increased from baseline of 3.2 (SD=1.1) to 3.9 (SD=1.1, p<0.001) at 18-month follow-up. Overall, increasing adherence to WCRF/AICR guideline was associated with higher scores of global health status (P=0.01), physical functioning (P<0.001) and role functioning (P=0.03), and lower scores of fatigue (P=0.001), nausea and vomiting (P=0.003), pain (P<0.001), dyspnea (P=0.006), loss of appetite (P=0.001) and diarrhea (P<0.001).

Conclusions: Positive lifestyle changes were made among Chinese breast cancer survivors after cancer diagnosis. Increased adherence to WCRF/AICR recommendations after cancer diagnosis improves QOL, suggesting that Chinese breast cancer survivors should follow the WCRF/AICR guideline for cancer prevention.

Acknowledgments: This study is funded by the World Cancer Research Fund International (Grant Number WCRF 2010/249 and WCRF 2014/1197) and Madam Diana Hon Fun Kong Donation for Cancer Research.
Title: Metastatic breast cancer collateral damage project (MBCCD): Development and preliminary results of the survey of health, impact, needs, and experiences (SHINE)

Annette Stanton¹, Timothy Williamson¹, Jessica Clague DeHart², Alexandra Jorge¹, Leah Eshraghi³, Heather Cooper Ortner³ and Susan Love³. ¹University of California, Los Angeles, Los Angeles, CA; ²City of Hope, Duarte, CA and ³Dr. Susan Love Research Foundation, Encino, CA.

Body: Rationale: People living with metastatic breast cancer face unique challenges, which have not been well characterized. The aim of the MBCCD was to characterize the MBC patient experience in order to identify and address potential areas of need in this underserved population.

Method: We crowdsourced input and gathered free-text data from 353 people living with MBC to document the breadth of challenges affecting quality of life. From the responses, we developed a new measure (SHINE) with 79 patient-driven items regarding the experience of MBC, including verbatim statements from crowdsourcing data. Validated quality of life scales, clinical information, and demographic data were also collected. Participants were recruited from Dr. Susan Love Research Foundation's Army of Women® and other advocacy organizations; 515 completed the questionnaire. Confirmatory and exploratory factor analyses were conducted to assess model fit for SHINE and to identify subscales. Next, differences in MBC-specific concerns and experiences as a function of age, marital status, financial status, education, children, metastatic site location(s), and current medical treatment were examined with ANOVAs and t-tests.

Results: Factor analyses indicated good model fit (CFI=.96, RMSEA=.05, SRMR=.04) for a 36-item scale of MBC-specific concerns and experiences with nine subscales: 1) Employment/achievement, 2) Finances, 3) Insurance, 4) Mortality/uncertainty, 5) Activity disruption, 6) Concern for others, 7) Social isolation/withdrawal, 8) Self-concept disruption, 9) Benefit finding. A within-subjects ANOVA revealed that participants were most bothered by mortality/uncertainty concerns, followed by activity disruption, financial, employment/achievement, and insurance concerns, respectively ($F(4,508)=111.38, p<0.01$). Additionally, participants endorsed strongest agreement with benefit finding, followed by concern for others, self-concept disruption, and social isolation/withdrawal ($F(4,508)=101.53, p<.01$). Participants younger than 50 years and those with lower financial status reported higher concerns on all subscales ($ps<0.01$), except for benefit finding ($ps>0.18$). Participants with a child under 18 living at home, those on combination therapies, and those with metastases to multiple sites or bone only reported higher concerns on several subscales ($ps<0.05$). Being married was significantly associated with higher concern for others and lower concern about finances ($ps<0.02$). Education was not significantly related to any subscale.

Conclusions: Several important concerns and experiences related to MBC are not captured adequately in existing measures. This study developed a new measure (SHINE), which reliably assessed these disease-specific concerns and experiences in 515 adults living with MBC. Concerns regarding mortality and uncertainty were most prominent. Specific demographic and medical characteristics modified responses on eight subscales, but not on the benefit finding subscale. Findings can aid the development of supportive care efforts that address areas of need (e.g., concerns about mortality and uncertainty) in this understudied medical population.
Title: Effect and moderators of exercise on fatigue in patients with breast cancer: Meta-analysis of individual patient data


1 University Medical Center Utrecht, Utrecht, Netherlands; 2 VU University Medical Center, Amsterdam, Netherlands; 3 University of Alberta, Edmonton, Canada; 4 Edith Cowan University, Joondalup, Australia; 5 Netherlands Cancer Institute, Amsterdam, Netherlands; 6 Moffitt Cancer Center and Research Institute, Tampa; 7 German Cancer Research Center (DKFZ) and National Center for Tumor Disease (NCT), Heidelberg, Germany; 8 Yale School of Public Health, New Haven; 9 Institute of Health and Biomedical Innovation, Queensland University of Technology, Kelvin Grove, Australia; 10 University of Maryland, Baltimore; 11 Maastricht University, Maastricht, Netherlands; 12 University of Groningen, Groningen, Netherlands; 13 Academic Medical Center, Amsterdam, Netherlands; 14 University of Edinburgh, Edinburgh, United Kingdom; 15 University of Birmingham, Birmingham, United Kingdom; 16 University of Glasgow, Glasgow, United Kingdom; 17 Heidelberg University, Mannheim, Germany; 18 Oslo University Hospital, Oslo, Norway; 19 University Medical Center Hamburg-Eppendorf, Hamburg, Germany; 20 University of Adelaide, Adelaide, Australia; 21 The University of Newcastle, Callaghan, Australia; 22 National Center for Tumor Diseases (NCT) and Heidelberg University Hospital, Heidelberg, Germany; 23 Netherlands Cancer Institute/Antoni van Leeuwenhoek Hospital, Amsterdam, Netherlands; 24 Penn State Health, College of Medicine, and Cancer Institute, Hershey; 25 Oregon Health & Science University, Portland; 26 Netherlands Comprehensive Cancer Organisation (IKNL), Utrecht, Netherlands and 27 Johns Hopkins School of Nursing, Johns Hopkins School of Medicine, Sidney Kimmel Comprehensive Cancer Center, Baltimore.

Body: Background - Fatigue is one of the most common and disabling complaints in patients with breast cancer and can effectively be reduced by physical exercise, with small to moderate effect sizes. To identify heterogeneity in responses to exercise and to further personalize exercise prescriptions, moderators of exercise effects on fatigue should be investigated. However, most randomized controlled trials (RCTs) are not adequately powered for such analyses. Therefore we conducted meta-analyses using the individual patient data of several exercise RCTs. The aim is to investigate the effect and moderators of physical exercise on cancer-related fatigue in patients with breast cancer.

Methods - Within the Predicting Optimal Cancer Rehabilitation and Supportive care (POLARIS) consortium, principal investigators of 34 exercise RCTs worldwide have shared their individual patient data. Twenty-two of these RCTs included patients with breast cancer with a total sample size of 3,061. Different questionnaires to assess level of fatigue were used, which was acknowledged by using z-scores in the analysis. A one-step individual patient data meta-analysis, using a linear mixed-effect model adjusted for baseline fatigue, with a random intercept on study (to account for study clustering) was undertaken to investigate effect of exercise on fatigue. The result, a between-group difference in z-scores, corresponds to a Cohen’s d effect size. An interaction term was included in the model to assess potential moderators including demographic (age, marital status, education), clinical (body mass index, presence of distant metastasis), intervention-related (intervention timing, delivery mode and duration), and exercise-related (exercise type, frequency, intensity, duration) characteristics.

Results – Exercise significantly reduced fatigue reported by women with breast cancer ($\beta = -0.15$, 95% CI $-0.21$; $-0.09$). This effect did not differ significantly between patients with different demographic and clinical characteristics ($p_{value_{interaction}} >0.05$). Also, neither timing (during or post-treatment) and duration of the intervention, nor exercise-related factors moderated intervention effects on fatigue. Supervised exercise had significantly larger effects on fatigue than unsupervised exercise ($\beta_{difference} = -0.17$, 95%CI $-0.28$; $-0.05$). Compared to the control group, supervised exercise significantly improved fatigue ($\beta = -0.21$, 95%CI $-0.28$; $-0.14$), while unsupervised exercise did not ($\beta = -0.04$, 95%CI $-0.14$; $0.06$).

Conclusion – Exercise significantly reduces fatigue in patients with breast cancer across subgroups formed on the basis of age, marital status, education level, body mass index, and presence of distant metastasis. The effect of exercise is significantly larger
when performed under supervision. Hence, exercise, and preferably supervised exercise, represents a viable intervention for the prevention and treatment of fatigue among patients with breast cancer.
Title: Metastatic breast cancer collateral damage project: Associations of disease-specific concerns and experiences with psychological health, illness management, and health behaviors

Timothy Williamson¹, Annette Stanton¹, Jessica Clague DeHart², Alexandra Jorge¹, Leah Eshraghi³, Heather Cooper Ortner³ and Susan Love³. ¹University of California, Los Angeles, Los Angeles, CA; ²City of Hope, Duarte, CA and ³Dr. Susan Love Research Foundation, Encino, CA.

Body: Rationale: Research has identified risk and protective factors that predict health and well-being in adults with breast cancer, but this work has been focused largely on women with early-stage disease. Metastatic breast cancer (MBC) patients report worse psychological health and poorer quality of life compared to those with early-stage breast cancer. We investigated whether a newly developed measure of MBC-specific concerns and experiences, the Survey of Health, Impact, Needs, and Experiences (SHINE), is associated with psychological health, illness management, and health behaviors in a sample of MBC patients.

Method: SHINE includes 36 items capturing the experience of MBC, including verbatim statements from previously collected crowdsourcing data. Participants (N=515) were recruited from Dr. Susan Love Research Foundation's Army of Women® and other advocacy organizations. Participants also completed measures of psychological health (i.e., depression, anxiety), illness management (i.e., self-efficacy for managing medications/treatments and symptoms), and health behaviors (i.e., sleep, degree of moderate physical activity). The nine SHINE subscales, along with age, marital status, financial status, children, metastatic site location(s), and current medical treatment(s), were entered as simultaneous predictors in a multivariate regression. Psychological health, illness management, and health behaviors were entered as dependent variables in separate models.

Results: With demographic and medical variables controlled, MBC-specific concerns were associated significantly with depressive symptoms (ΔR²=.38), anxiety (ΔR²=.37), efficacy for medication/treatment management (ΔR²=.08), efficacy for symptom management (ΔR²=.32), sleep disruption (ΔR²=.10), and physical activity (ΔR²=.17), with all ps<.01. Specifically, higher depressive symptoms and anxiety were associated greater mortality/uncertainty concerns (ps<.01), greater social isolation/withdrawal (ps<.01), and higher self-concept disruption (ps<.02). Anxiety was uniquely associated with greater financial concerns (p=.02), whereas depression was uniquely associated with fewer positive experiences (p<.05). Higher self-efficacy for managing symptoms was related to more positive experiences, higher mortality concerns, and more self-concept and activity disruption (p=.01), whereas higher efficacy for managing medications/treatments was associated with lower social isolation/withdrawal (p=.03). More sleep disruption was associated with higher concern for others (p=.05), and less physical activity was associated with more activity disruption (p=.01).

Conclusions: After controlling for demographic and medical characteristics, MBC-specific concerns were related significantly to psychological health, self-efficacy for illness management, and health behaviors. Mortality/uncertainty concerns, social isolation/withdrawal, and self-concept disruption were especially important correlates of depressive symptoms, anxiety, and MBC-related self-efficacy. Higher concern for others was related to more sleep disruption. Approaches that address these MBC-specific concerns and promote positive experiences may be beneficial for patients.
2017 San Antonio Breast Cancer Symposium

Publication Number: P6-12-08

Title: Fertility interest, management and outcomes in young BRCA+ breast cancer survivors

Philip D Poorvu1, Shari I Gelber1, Kathryn J Ruddy1, Kira Seiger1, Rulla M Tamimi2, Jeffrey Peppercorn3, Lidia Schapira4, Virginia F Borges5, Steven E Come6, Ann H Partridge1 and Shoshana M Rosenberg1. 1Dana-Farber Cancer Institute, Boston, MA; 2Brigham and Women’s Hospital, Boston, MA; 3Massachusetts General Hospital, Boston, MA; 4Stanford University, Stanford, CA; 5University of Colorado Cancer Center, Aurora, CO and 6Beth Israel Deaconess Medical Center, Boston, MA.

Background: Young women with BRCA mutations may face fertility issues given the standard recommendation for risk-reducing oophorectomy after childbearing has been completed or before age 40. Potential transmission of the affected gene to future progeny may also be a concern. Little is known regarding the perspectives, management, and outcomes of young breast cancer survivors with BRCA mutations, who also face risks of recurrent disease and treatment effects on fertility.

Methods: As part of a multi-center, prospective cohort study of newly diagnosed breast cancer (BC) at age ≤40 years enrolling between 2006-2016, we identified women with stage I-III BC who had self-reported results of genetic testing. Participants are surveyed at baseline then annually regarding their breast cancer treatment, genetic testing, fertility interest, pregnancy attempts, and pregnancies. Chi-square tests were used to compare proportions of carriers vs non-carriers who were interested in future biologic children, took steps to preserve fertility, underwent bilateral oophorectomy, attempted pregnancy, and became pregnant in the 5 years following diagnosis.

Results: Carriers (n=104) and non-carriers (n=662) were similar in age and stage, but greater proportions of carriers had ER negative disease and received chemotherapy (Table 1). The proportion of carriers and non-carriers interested in future biologic children was similar prior to diagnosis (51% vs 38%; p=0.18), 1 year following diagnosis (30% vs 27%; p=0.44), and 5 years following diagnosis (14% vs 15%; p=0.26). Similar proportions of carriers (12%) and non-carriers (14%) took steps to prevent infertility prior to treatment. Greater proportions of carriers indicated that concern about having a child at higher risk of breast cancer affected their interest in future biologic children (15% vs 4%, p=0.02) and underwent bilateral oophorectomy (61% vs 9%, p<0.0001), but there was no difference in rates of pregnancy attempts (15% vs 11%, p=0.62), or pregnancies (12% vs 8%, p=0.36) in the five years following diagnosis.

Conclusion: Young breast cancer survivors with known BRCA mutations have similar interest in future fertility and both attempt and become pregnant at similar rates to non-carriers in the five years following diagnosis. Impact of specific BRCA mutation (1 or 2), ER status of tumor, and timing of pregnancy attempts will be explored in future analyses.

Table 1:

<table>
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<tr>
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<th>BRCA mutation carriers, n (%)</th>
<th>Non-carriers, n (%)</th>
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<td></td>
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<tr>
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<tr>
<td>31-35</td>
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<td>201 (30)</td>
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<tr>
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<td>375 (57)</td>
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<tr>
<td>Stage</td>
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<td>40 (39)</td>
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<td>46 (44)</td>
<td>307 (46)</td>
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<tr>
<td>III</td>
<td>18 (17)</td>
<td>95 (14)</td>
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<tr>
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<td>0.44</td>
</tr>
<tr>
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<td>77 (74)</td>
<td>509 (77)</td>
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</tr>
<tr>
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<td>148 (22)</td>
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</tr>
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<td>5 (1)</td>
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<tr>
<td></td>
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<td>No</td>
<td>Children pre-diagnosis</td>
</tr>
<tr>
<td>----------------</td>
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<td>--------</td>
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</tr>
<tr>
<td></td>
<td>62 (60)</td>
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<td>Adjuvant hormones</td>
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<tr>
<td></td>
<td>427 (64)</td>
<td>235 (36)</td>
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</table>
**Body:** **Background:** Due to early detection and improved treatments, women with breast cancer are living longer. Breast cancer shares risk factors with cardiovascular disease (CVD), and its treatments have adverse cardiovascular effects. Less is known about the association between cardiac risk factors and long-term cardiac events among the patients enrolled in breast cancer trials, as most trials fail to collect this information.

**Methods:** We examined the SWOG database to identify phase II/III breast cancer trials from 1999-2011. Among patients over 65 years, we linked the SWOG clinical records to Medicare claims data according to social security number and date of birth. This analysis included patients with 6+ months of Medicare coverage prior to baseline and 12+ months of Medicare coverage at any point after baseline. The comorbidities investigated at baseline were diabetes, hypertension, hypercholesterolemia, coronary artery/ischemic heart disease and obesity. A cardiac event was defined as an acute ischemic event or acute heart failure. Cox regression was used to calculate time-to-event, stratified by study ID and adjusted for baseline age, race, and prognostic risk score. Cox regression was performed separately for each CVD risk factor, and an additional analysis was performed to assess the impact of having multiple concurrent risk factors. Secondary analyses were performed separately by study type (Adjuvant, Advanced).

**Results:** Among patients linked to Medicare included in this cohort (N=742), the median age was 70, and median follow-up was 6 years. The majority of patients were non-Hispanic white. The most prevalent conditions were hypercholesterolemia (58%) and hypertension (73%). Only 13% had no baseline risk CVD factors. In a Cox regression, all baseline risk factors except hypercholesterolemia and obesity were statistically significantly or borderline statistically significantly associated with an increased risk of eventual cardiac event, and for ischemic heart disease the increased risk was more than two-fold (HR=2.27, 95% CI=1.46-3.54, p=0.0003) and for baseline diabetes nearly two-fold (HR=1.75, 95% CI=1.13-2.71, p=0.01). In addition, there was evidence of a linear association of number of concurrent risk factors and cardiac events (HR per additional risk factor = 1.35 (1.09-1.66), p=0.005). In the stratified analysis, the associations were statistically significant only for participants on adjuvant studies. No association between baseline cardiac risk factors and cardiovascular outcomes were seen among patients with advanced cancer.

**Conclusions:** In summary, we found that even among healthy breast cancer patients selected for clinical trials, baseline CVD risk factors are associated with an increased risk of cardiac events, however this association was not observed for patients with advanced disease, who are more likely to die from breast cancer before experiencing a cardiovascular event.
2017 San Antonio Breast Cancer Symposium

Publication Number: P6-12-10

Title: The importance of a survivorship coordinator role in the creation of survivorship care plans and maintaining compliance with new accreditation standards

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Body: Background: Froedtert Hospital and the Medical College of Wisconsin's Breast Care Center (F&MCWBCC), is a Commission on Cancer (CoC) and National Accreditation Program for Breast Centers (NAPBC) accredited facility providing care to over 500 patients a year. The F&MCWBCC recognized the importance of following the IOM survivorship recommendations and in 2014 began providing a survivorship care plan (SCP) to patients completing radiation therapy. In 2015, SCP standards for both CoC and NAPBC were established. A redesign of the process was necessary to meet these new requirements.

Methods: A review of accreditation standards was conducted along with an examination of staff availability. A collaborative team of content experts was gathered who worked with an informatics nurse to design content and a template in the EHR that met the elements outlined by the accreditation standards. Initially, treating physicians and advanced practice nurses were responsible for creating the SCP. However, these providers found it to be increasingly difficult to manage on top of their other responsibilities. In May 2015, an experienced breast cancer technician was identified for the survivorship data coordinator role. The coordinator used available sources, patient lists and clinic schedules, to case find. She developed spreadsheets to assist in tracking patients and measuring compliance. The coordinator communicated with providers to alert them to populate and review the SCP and coordinated the survivorship appointment with the nurse.

Once the SCP is completed, a dedicated RN position is utilized for a survivorship visit with patients. During this visit, the RN reviews the care plan and provides education on follow up care guidelines and overall health and wellness concepts. Assessments for further supportive services are performed, and appropriate referrals made. The RN shares the SCP with the primary care provider. Results are tracked and reported to Cancer Service Line Leadership and the Breast Steering and Cancer Committees.

Results: During the implementation phase, completion of SCPs by physicians and advance practice nurses was inconsistent. Once the survivorship coordinator role was created and implemented, compliance with NAPBC standards improved as SCP delivery numbers increased 141%.

The survivorship coordinator improved delivery of SCPs. In 2014, 130 plans were delivered, and 185 in 2015. In 2016, 314 were delivered, achieving 100% compliance to the NAPBC survivorship standard.

Conclusion: Compliance with NAPBC and CoC standards to deliver SCPs to patients and primary care providers is labor intensive and requires a large number of resources. Use of a survivorship data coordinator has proven successful in determining and tracking eligible patients and ensuring timely delivery of SCPs to patients. The monitoring of new patients through this process has enabled this center to go from estimates of analytic cases, to a current real-time numbers. This is necessary to provide a comprehensive review of care and survivorship guidelines to cancer survivors and meeting accreditation standards. Further work is needed to assess the impact of the SCPs on patient outcomes and satisfaction.
Title: Feasibility and biomarker modulation due to high levels of moderate to vigorous physical activity as part of a weight loss intervention in older, sedentary, obese breast cancer survivors

Carol J Fabian1, Jennifer R Klemp1, Jeffrey M Burns1, Eric D Vidoni1, Jennifer L Nydegger1, Amy L Kreutzjans1, Teresa L Phillips1, Hailey A Baker1, Bill Hendry1, Casey John1, Amanda L Amin1, Qamar J Khan1, Melissa P Mitchell1, Anne P O’Dea1, Priyanka Sharma1, Jamie L Wagner1, Stephen D Hursting2 and Bruce F Kimler1. 1University of Kansas Medical Center, Kansas City, KS and 2University of North Carolina, Chapel Hill, NC.

Body: We sought to demonstrate that older, sedentary, obese breast cancer survivors could achieve > 200 minutes per week of moderate to vigorous physical activity (MVI PA) as part of a weight loss intervention; and to assess modulation of risk biomarkers. This level of PA in combination with moderate calorie restriction is associated with weight losses of >10% in women without cancer, which in turn is associated with significant modulation of cancer risk biomarkers.

Eleven participants with BMI > 30 kg/m² enrolled in a 12-week program that consisted of moderate caloric restriction, weekly phone group behavioral sessions, and individualized exercise plans based on measured heart rate reserve. Women were provided an accelerometer with heart rate monitor linked to GarminConnect, membership to a YMCA, twice weekly supervised exercise sessions with a personal trainer, and weekly feedback regarding weight and physical activity progress. The goal was to increase MVI PA (≥45% heart rate reserve) gradually from <60 to >200 minutes per week.

The median age was 61, 5/11 women had received prior chemotherapy, and 7/11 were currently taking aromatase inhibitors. Median values of baseline anthropomorphic measures acquired by dual energy x-ray absorptiometry (GE Lunar iDXA) included BMI, 37.3 kg/m²; total mass, 97.5 kg; fat mass, 47.6 kg; visceral fat, 1.7 kg (range 1.4-3.0); and fat mass index, 17.6 kg/m². The majority had a baseline VO2 peak in the poor range for their age. All 11 participants completed the intervention, with no reported serious adverse events. Median MVI PA achieved over weeks 5-12 was 161 minutes/week (range 48-320). VO2 peak was increased in 10/11 with a median relative change of 12% from baseline. All but one lost weight with an overall median of 8% total mass loss, which was associated with 13% total fat mass loss and 21% visceral fat mass loss. For those with MVI PA above the median, values were 11%, 17%, and 40%, respectively. Visceral fat mass loss was linearly correlated with minutes per week of MVI PA (p=0.032); these parameters in turn were associated with changes in a number of serum biomarkers, including adiponectin-leptin ratio, TNF-alpha, as well as circulating adipose stromal cells, a potential marker for metastasis. Insulin and hs-CRP were favorably modulated in almost all participants but change was not linearly correlated with activity or mass loss parameters; thus these may not be ideal biomarkers to document a dose response to level of MVI PA.

Conclusion: These results demonstrate that older, sedentary, obese breast cancer survivors can safely achieve a high level of MVI PA when provided a structured program that includes an exercise trainer. It is feasible to design a clinical trial for such breast cancer survivors to examine biomarker modulation as a function of level of physical activity.
Title: Improvement in sexual function over time in premenopausal women with breast cancer

Shari B Goldfarb1, Sabrina Kamer1, Raymond Baser1, Jessica Quistorff1, Mary L Gemignani1 and Maura Dickler1. 1Memorial Sloan Kettering Cancer Center, New York, NY.

Body: Background: There is evidence that many cancer survivors live with sexual dysfunction that impacts their quality of life. It is essential to identify factors that influence the development of sexual symptoms and understand their trajectory over time in order to guide potential interventions to treat sexual dysfunction. Most studies to date have been cross-sectional and longitudinal studies are needed to understand the change of sexual function over time. This study aims to investigate and describe the factors that impact sexual health and dysfunction in breast cancer patients during and after their cancer treatment.

Methods: A longitudinal prospective trial is being conducted in premenopausal women 18-50 years of age with breast cancer being treated at MSKCC. Validated questionnaires on sexual health and function were administered to patients after they were diagnosed with breast cancer, but before they initiated cancer treatment and at one-year follow-up after initiation of primary breast cancer therapy. Demographic and treatment information was also collected. The female sexual function index (FSFI) total and individual domain scores were calculated. Baseline and 12-month scores were compared using paired t-tests. Multivariable linear regression was used to assess individual variable associations with 12-month FSFI total scores controlling for baseline scores.

Results: 127 women were eligible for analysis at the time of this abstract and had a median age of 41. Eighty-nine percent of tumors were estrogen receptor positive and 24.4% were HER-2 overexpressing. Eighty-nine percent of patients received chemotherapy, 61.4% received Tamoxifen and 23% received a LHRH agonist in combination with an aromatase inhibitor. Mean FSFI total score was 20.4 at baseline and 21.2 at 12-months post diagnosis. More than half of women met FSFI criteria for sexual dysfunction (FSFI score<26) at baseline (57.5%) and 12-months (55.2%). Small increases in sexual activity were seen with 27.8% of patients inactive at baseline compared to 23.2% at 12 months. Similarly, women engaging in sexual activity more than once a week increased from 9.5% to 16.8%. Desire (libido) significantly improved (p = 0.023) from baseline to 12 months. Controlling for baseline score, younger age and treatment with tamoxifen were associated with better 12-month scores (p < 0.05).

Conclusions: Mean FSFI scores in our patients with breast cancer before and after treatment are consistent with scores from other studies looking at cancer patients and are lower than those of healthy women. In the peri-diagnosis period patients had worse sexual function that showed signs of small improvements 12 months after initiation of treatment, especially in the desire domain. Patients are being followed to see if sexual function continues to improve over time, to better understand the factors causing sexual dysfunction in these patients and to determine the best time to intervene in order to improve symptoms.
Title: Developing a non-hormonal treatment for vaginal dryness for breast cancer survivors: A pilot study of a therapeutic ultrasound device

Shannon MacLaughlan David¹, Holly Rockweiler¹,², Ryan Krone¹,², Stephanie Middelton¹ and Douglas Blayney¹. ¹Stanford University and ²Madorra Inc.

Body: Objectives: Breast cancer survivors need a non-hormonal treatment for vaginal dryness, as estrogen replacement therapy is often contraindicated or undesired. Therapeutic ultrasound applied to the vaginal introitus is safe and was shown to increase vaginal temperature and blood flow in our phase I study. We now report results from a twelve-week trial of daily, self-applied therapeutic ultrasound to the vaginal introitus.

Methods: Breast cancer survivors and post-menopausal women with symptomatic vaginal atrophy were enrolled. A gynecologic oncologist supervised participants in application of a gel-pad equipped ultrasound head (Intelect TranSport, Chattanooga Group) to the vaginal introitus at an enrollment visit, and instructed women on self-application. Daily, 8-minute treatment applications for 12 weeks were planned, and dose was titrated as needed for comfort. Vaginal Maturation Index (VMI) specimens were collected and Vaginal Health Index (VHI) was recorded at study visits. Patient-reported outcomes for vaginal dryness and personal lubrication were recorded on a Likert-type scale (0-3). Student's t-test was used to analyze ordinal and continuous variables in an intent-to-treat analysis.

Results: From December 2015 to January 2017, 20 women were enrolled, including 7 breast cancer survivors. Mean VMI for the study population was 25.1 (median 25) at baseline, and 21.4 (median 6) after 12 weeks of treatment (p>0.05). Similarly, there were no significant changes seen in mean VHI, which was 12.8 (median 13) at baseline and 14.1 (median 14) at 12 weeks (p>0.05). Statistically significant improvements were seen in both vaginal dryness and lubrication as reported by patients' scores. The mean vaginal dryness score for the population was 1.9 (median 2) at baseline and 1 (median 1) at 12 weeks (p<0.05). The effect was more pronounced in a subset of women (n=6) who did not use ultrasound jelly with their device (mean baseline score of 2.3 reduced to 1 at 12 weeks). Baseline vaginal lubrication scores (mean 0.6, median 1) for the population also improved after 12 weeks (mean 1.4, median 1, p<0.05), though the scores remained in the “mild” range. The six women who used the ultrasound device without jelly reported essentially no lubrication at the start of the study (mean 0.17, median 0), and had notably improved symptoms, reporting moderate lubrication after 12 weeks (mean and median score 1.5). Of the 15 women who completed the treatment according to protocol, 93% reported an improvement in at least one of their symptoms.

Conclusions: Self-application of therapeutic ultrasound to the vaginal introitus decreased symptoms of vaginal atrophy in the majority of users. While no detectable changes in tissue physiology were noted with the VMI or VHI tools, the notable improvement in patient-reported outcomes warrants further study. A phase III clinical trial with a customized device is planned.
2017 San Antonio Breast Cancer Symposium

Publication Number: P6-12-14

Title: Quality of life and ability to work in patients at different disease stages of HER2+ breast cancer

Mark Verrill¹, Andrew Wardley², Jenny Retzler³, Adam B Smith⁴, Donna McNicol⁵, Sorcha Dando⁴, Irwin Tran⁵, Iain Leslie⁶ and Peter Schmid⁶. ¹The Newcastle Upon Tyne Hospitals NHS Foundation, Newcastle, United Kingdom; ²The Christie NHS Foundation Trust, Manchester, United Kingdom; ³York Health Economics Consortium, York, United Kingdom; ⁴pH Associates Ltd (an Open Health Company), Marlow, United Kingdom; ⁵Roche Products Ltd, Welwyn Garden City, United Kingdom and ⁶Queen Mary University of London, London, United Kingdom.

Body: OBJECTIVES: Health-related quality of life (HRQoL) and ability to work in patients treated for HER2+ early breast cancer (EBC) are poorly understood. This study compared HRQoL and ability to work in 3 HER2+ patient cohorts: EBC during adjuvant treatment, EBC after treatment, and metastatic disease (MBC).

METHODS: A cross-sectional observational cohort study of 299 female consenting patients with HER2+BC, from 14 UK secondary care centres. Group1 (n=89): receiving targeted HER2 therapy±chemotherapy for EBC; Group2 (n=108): in follow up post-targeted treatment for eBC; Group3 (n=102): MBC on treatment. Data collected between Dec 2016-Mar 2017: HRQoL, demographic and employment status data collected via patient-reported questionnaires (including EQ-5D-5L and Functional Assessment of Cancer Therapy [FACT-B]); clinical data collected from medical records. Inter-group differences were assessed using univariate Analysis of variance (ANOVA) and chi-square tests as appropriate. [NCT03099200].

RESULTS: Table1 shows patient demographics, disease characteristics, employment status, and EQ-5D-5L scores. Group1 and Group2 patients did not differ in overall health utility or visual analogue scale (VAS) scores. However, Group3 patients reported significantly poorer health utility than Group1 (p<0.02) and Group2 (p<0.001), and significantly worse VAS scores than Group2 (p<0.001). Significantly fewer Group2 patients and more Group3 patients were unable to work (p<0.003), and fewer Group3 patients were employed than expected (by chi-square, p<0.003).

CONCLUSIONS: HRQoL in patients with EBC was similar whether on or off treatment, and better than those with MBC. HRQoL scores reported on the generic EQ-5D will be compared with those from the disease-specific FACT-B. A smaller proportion of patients with MBC were employed compared to the EBC groups, reflecting the impact of advanced disease. Fewer patients with EBC reported being unable to work than we expected, suggesting these patients maintain function.

Table1

<table>
<thead>
<tr>
<th></th>
<th>Group1 (n=89)</th>
<th>Group2 (n=108)</th>
<th>Group3 (n=102)</th>
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<td>55 (11)</td>
<td>55 (11)</td>
</tr>
<tr>
<td><strong>Hormone receptor status¥</strong></td>
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<tr>
<td>Positive</td>
<td>64 (72%)</td>
<td>84 (78%)</td>
<td>74 (73%)</td>
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<tr>
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<td>25 (28%)</td>
<td>24 (22%)</td>
<td>26 (26%)</td>
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<tr>
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<td>0 (0%)</td>
<td>0 (0%)</td>
<td>2 (2%)</td>
</tr>
<tr>
<td><strong>Time since diagnosis (months)+</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EBC</td>
<td>9 (6)</td>
<td>45 (32) (n=103)</td>
<td>80 (82) (n=71*)</td>
</tr>
<tr>
<td>MBC</td>
<td>-</td>
<td>-</td>
<td>39 (36) (n=101)</td>
</tr>
<tr>
<td><strong>Employment status¥</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employed</td>
<td>45 (51%)</td>
<td>55 (51%)</td>
<td>28 (28%) $</td>
</tr>
<tr>
<td>Not employed</td>
<td>41 (46%)</td>
<td>52 (48%)</td>
<td>69 (68%)</td>
</tr>
<tr>
<td>Retired</td>
<td>22 (25%)</td>
<td>39 (36%)</td>
<td>33 (32%)</td>
</tr>
<tr>
<td>Unable to work</td>
<td>7 (8%)</td>
<td>5 (5%) $</td>
<td>27 (27%) $</td>
</tr>
<tr>
<td>Other</td>
<td>12 (14%)</td>
<td>8 (7%)</td>
<td>9 (9%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>3 (3%)</td>
<td>1 (1%)</td>
<td>5 (5%)</td>
</tr>
</tbody>
</table>
### EQ-5D summary scores

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual analogue scale</td>
<td>72.7 (18.4)†</td>
<td>77.0 (17.5)†</td>
<td>65.8 (22.9)† (n=99)</td>
</tr>
<tr>
<td>Utility value</td>
<td>0.809 (0.170)† (n=86)</td>
<td>0.818 (0.181)†</td>
<td>0.695 (0.262)† (n=97)</td>
</tr>
</tbody>
</table>

†mean (standard deviation); ¥n (%); +median (interquartile range); *excludes 27/102 patients (27%) with de novo MBC. **Bold text**: observed differences between three groups at significance thresholds of ‡p<0.05, §p<0.003 or †p<0.001. EBC/MBC: early/metastatic breast cancer. %s have been rounded so may not total 100%.
Title: The psychosocial impact of surgical scars in survivorship: Findings from a nationwide survey of women breast cancer survivors

Jennifer S Gass¹, Sunny Mitchell² and Michael Hanna³. ¹Women and Infants’ Hospital, Providence, RI; ²Westchester Medical Center, Valhalla, NY and ³Mercury Medical Research & Writing, New York, NY.

Body: Background: Surgery is an integral component of comprehensive breast cancer therapy, but leaves physical scars that may have psychosocial consequences in survivorship. Previous studies have shown that breast cancer treatment has a negative impact on body image, sexual function, mental health and social adjustment. As overall survival from early staged breast cancer approaches 99%, more data is needed on the late and long term consequences of breast cancer treatment and quality of life in survivorship. The aim of this research was to explore how women are affected specifically by physical scars from breast cancer surgery.

Methods: A nationwide internet survey was conducted among women who reported being surgically treated by lumpectomy only (n=215), mastectomy only (n=140), or both procedures (n=132) for breast cancer. To improve generalizability, census-based enrollment quotas were applied for geographic region, health insurance, and income.

Results: A solid majority of women in each of the three study groups agreed somewhat or strongly that they “do not like the location of my surgical scar” (table 1). Younger women were more likely to agree strongly with that statement (table 2).

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>%</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lumpectomy only (n=215)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agree strongly</td>
<td>43</td>
<td>20</td>
<td>15</td>
<td>25</td>
</tr>
<tr>
<td>Agree somewhat</td>
<td>94</td>
<td>44</td>
<td>38</td>
<td>50</td>
</tr>
<tr>
<td>Disagree somewhat</td>
<td>46</td>
<td>21</td>
<td>16</td>
<td>27</td>
</tr>
<tr>
<td>Disagree strongly</td>
<td>32</td>
<td>15</td>
<td>11</td>
<td>21</td>
</tr>
<tr>
<td><strong>Mastectomy only (n=140)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agree strongly</td>
<td>46</td>
<td>33</td>
<td>24</td>
<td>41</td>
</tr>
<tr>
<td>Agree somewhat</td>
<td>47</td>
<td>34</td>
<td>26</td>
<td>42</td>
</tr>
<tr>
<td>Disagree somewhat</td>
<td>33</td>
<td>24</td>
<td>16</td>
<td>31</td>
</tr>
<tr>
<td>Disagree strongly</td>
<td>14</td>
<td>10</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td><strong>Both surgeries (n=132)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agree strongly</td>
<td>31</td>
<td>24</td>
<td>17</td>
<td>31</td>
</tr>
<tr>
<td>Agree somewhat</td>
<td>89</td>
<td>67</td>
<td>58</td>
<td>75</td>
</tr>
<tr>
<td>Disagree somewhat</td>
<td>10</td>
<td>8</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>Disagree strongly</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 2. Regression Analyses for Strong Agreement with the Statement, “I do not like the location of my surgical scar”.

Results for the Main Outcome, “I do not like the location of my surgical scar”.
<table>
<thead>
<tr>
<th></th>
<th>OR</th>
<th>lower 95% CI</th>
<th>Upper 95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumpectomy only (n=215)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.96</td>
<td>0.94</td>
<td>0.999</td>
<td>0.010</td>
</tr>
<tr>
<td>Significant Other</td>
<td>2.3</td>
<td>0.86</td>
<td>6.3</td>
<td>0.098</td>
</tr>
<tr>
<td>Income</td>
<td>0.9</td>
<td>0.7</td>
<td>1.2</td>
<td>0.5</td>
</tr>
<tr>
<td>College Graduate</td>
<td>0.7</td>
<td>0.3</td>
<td>1.8</td>
<td>0.5</td>
</tr>
<tr>
<td>Mastectomy Only (n=140)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>College Graduate</td>
<td>0.3</td>
<td>0.1</td>
<td>0.7</td>
<td>0.006</td>
</tr>
<tr>
<td>Age</td>
<td>0.97</td>
<td>0.94</td>
<td>0.99</td>
<td>0.04</td>
</tr>
<tr>
<td>Income</td>
<td>0.8</td>
<td>0.6</td>
<td>1.2</td>
<td>0.4</td>
</tr>
<tr>
<td>Significant Other</td>
<td>1.2</td>
<td>0.6</td>
<td>2.7</td>
<td>0.6</td>
</tr>
<tr>
<td>Both Surgeries (n=132)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>College Graduate</td>
<td>15</td>
<td>5.0</td>
<td>44</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Significant Other</td>
<td>0.6</td>
<td>0.2</td>
<td>1.9</td>
<td>0.4</td>
</tr>
<tr>
<td>Age</td>
<td>0.99</td>
<td>0.94</td>
<td>1.03</td>
<td>0.5</td>
</tr>
</tbody>
</table>

In each of the three study groups, the majority of women agreed that they did not realize how uncomfortable the scars would make them feel when undressed or when seen undressed by someone else. The majority of women in each of the three study groups also felt self-conscious some or all of the time due to the surgical scars and avoided certain pieces of clothing some or all of the time because they revealed the scars.  

**Conclusion:** Consistent with previous literature, this survey shows that surgical scars from lumpectomy and mastectomy are not merely “cosmetic” but have a substantial impact on the lives and well-being of women in survivorship. Surgeons may be able to improve the breast survivorship journey for women by employing techniques that minimize visibility of surgical scars. Efforts to minimize the iatrogenic impact of surgery and provide comprehensive care may ensure good psychosocial survivorship.
L-Dex surveillance of breast cancer-related lymphoedema: A retrospective study

Louise A Koelmeyer\textsuperscript{1}, Robert Borotkanics\textsuperscript{2}, Caleb J Winch\textsuperscript{1}, Philip Prah\textsuperscript{2}, Jessica M Alcorso\textsuperscript{1}, Kristine Nakhel\textsuperscript{1} and John Boyages\textsuperscript{1}. \textsuperscript{1}Macquarie University, Sydney, NSW, Australia and \textsuperscript{2}Auckland University of Technology, Auckland, New Zealand.

**Body: Objectives:** Breast cancer-related lymphoedema involves chronic, progressive, and incurable swelling in the treated breast or ipsilateral arm, hand, and/or trunk. The traditional referall-based model of care involves women being referred to a lymphoedema therapist after the onset of symptoms. Clinical guidelines from the United States, United Kingdom, and Australia have urged that lymphoedema surveillance and early intervention be implemented routinely after breast cancer treatment. Bioimpedance spectroscopy (BIS) is a candidate diagnostic modality for lymphoedema surveillance because BIS may measure the accumulating extracellular fluid that is characteristic of early lymphoedema. This cohort study aimed to determine whether prospective surveillance using BIS resulted in earlier detection and effective management of lymphoedema.

**Method:** The study cohort were women with breast cancer referred to a single lymphoedema therapist at a private multidisciplinary practice in Sydney, Australia. Data was collected prospectively between 1 January 2007 and 31 December 2016, during which 824 women were measured using an ImpediMed L-Dex\textsuperscript{®} U400 BIS device. Women were defined as surveillance if monitoring began pre-surgery (\( n = 292/824 \)) or within 90 days post-surgery (\( n = 148/824 \)) and continued for at least 90 days thereafter. Women were defined as referrals if monitoring began after 90 days post-surgery (\( n = 318/824 \)). Lymphoedema was diagnosed if BIS indicated extracellular fluid levels had increased by >10 L-Dex points from a woman's pre-surgical baseline, or exceeded the normative range by >10 L-Dex points or were maintained below these levels only by ongoing compression use. International Society of Lymphology stage at diagnosis was recorded. Swelling within 90 days of surgery or 270 days of commencing taxane-based chemotherapy was not defined as lymphoedema.

**Results:** Patient chart data was analysed retrospectively. The L-Dex measurements in the referral group were taken significantly longer post-surgery (\( Med = 784 \) days, \( IQR = 356-1977 \)) than the surveillance group (\( Med = 124 \) days, \( IQR = 104-188 \), \( p < 0.001 \)). A higher proportion of women in the surveillance group were diagnosed at the subclinical and mild stages of lymphoedema (Stage 0 and Stage 1) in comparison to women in the referral group who were more likely to be diagnosed in the moderate or severe stages of lymphoedema (Stage 2 and Stage 3). Higher median L-Dex values were found for women in the referral group (\( Med = 4.3 \), \( IQR = -0.8-13.6 \)) than those in the surveillance group (\( Med = 2.2 \), \( IQR = -1.9-5.8 \), \( p < 0.001 \)).

**Conclusion:** Prospective surveillance may result in earlier intervention with L-Dex measurements, earlier diagnosis of lymphoedema and lower L-Dex values. Ongoing statistical analyses will inform the clinical risk factors leading to increased lymphoedema incidence. This study has important implications for breast cancer clinical practice guidelines.
Title: Identifying risk factors and effect modifiers of trastuzumab-induced cardiotoxicity among multi-ethnic women with early-stage HER2-positive breast cancer

Ariel Yuan\textsuperscript{1}, Veli Topkara\textsuperscript{2}, Dawn L Hershman\textsuperscript{1,2,3}, Kevin Kalinsky\textsuperscript{2,3}, Melissa K Accordino\textsuperscript{2,3}, Meghna S Trivedi\textsuperscript{2,3}, Anqi Yu\textsuperscript{1,3}, Jeanine M Genkinger\textsuperscript{1,3} and Katherine D Crew\textsuperscript{1,2,3}. \textsuperscript{1}Columbia University Medical Center, New York, NY; \textsuperscript{2}Columbia University Medical Center, New York, NY and \textsuperscript{3}Herbert Irving Comprehensive Cancer Center, Columbia University Medical Center, New York, NY.

Body: Introduction: Trastuzumab-based adjuvant therapy is the current standard of care for early-stage HER2-positive breast cancer. However, trastuzumab has also been associated with an increased risk of cardiotoxicity, especially when given following an anthracycline. Trastuzumab-induced cardiotoxicity (TIC) can present as asymptomatic left ventricular ejection fraction (LVEF) decline or symptomatic heart failure. Our objective was to identify predictors of TIC among multi-ethnic patients with early-stage HER2-positive breast cancer. Unlike prior observational studies, our study included a high representation of racial/ethnic minorities, who are at increased risk of cardiovascular disease (CVD) compared to non-Hispanic whites.

Methods: We conducted a retrospective cohort study in patients with stage I-III HER2-positive breast cancer, diagnosed from 2007 to 2015 at Columbia University Medical Center (CUMC) in New York, NY, who had received adjuvant trastuzumab therapy. Participants had at least two serial echocardiograms or MUGA scans to assess TIC, which was defined as at least a 10% decrease in LVEF from baseline or LVEF <50%. LVEF recovery was defined as at least a 10% increase in LVEF or LVEF >50%. We conducted descriptive statistics and univariate and multivariable logistic regression to estimate the associations between socio-demographic factors, breast tumor and treatment characteristics, and CVD risk factors (including smoking status, body mass index [BMI], hypertension, diabetes, hyperlipidemia, coronary artery disease) and TIC. Interactions between race/ethnicity and CVD risk factors were assessed using a logistic regression model.

Results: In our study population (N=279), the mean age was 52.7 years (standard deviation, 12.1) with 36.6% non-Hispanic white, 18.3% non-Hispanic black, 34.8% Hispanic, and 10.4% Asian patients. There were no differences by race/ethnicity in tumor and treatment characteristics (over half had prior anthracyclines), but racial/ethnic minorities had higher BMI and were more likely to have hypertension compared to non-Hispanic whites. About a third of patients developed TIC and 14.7% had an LVEF decline to <50%, of which 15 (16.1%) experienced LVEF recovery. In multivariable analysis, prior anthracycline use and hypertension were significantly associated with increased odds of developing TIC (odds ratio [OR]: 2.25, 95% confidence interval [CI]: 1.25, 4.06; OR: 2.13, 95% CI: 1.15, 3.93, respectively). There was a significant interaction (p=0.027) between race/ethnicity and hypertension on odds of developing TIC with hypertensive non-Hispanic white patients experiencing 6.05 (95% CI: 2.19, 16.75) times the odds of developing TIC compared to non-hypertensive non-Hispanic whites.

Discussion: We observed a higher incidence of TIC and lower incidence of LVEF recovery compared to previous clinical trials. Given patient selection for clinical trials, our results may be more representative of clinical practice settings. We found a particularly high risk among non-Hispanic white patients with hypertension. Patients with hypertension may require closer blood pressure monitoring and treatment with anti-hypertensives in order to reduce risk of developing cardiotoxicity.
2017 San Antonio Breast Cancer Symposium

Publication Number: P6-12-18

Title: CANTOCHEM: Analysis of chemotherapy practice and early side effects in the 6090 first patients from the prospective CANTO cohort

Paul H Cottu¹, Yael Amar¹, Barbara Pistilli², Hélène Bonsang-Kitzis¹, Anne Lesur³, Florence Lerebours⁴, Laurence Vanlemmens⁵, Olivier Tredan⁶, Christelle Levy⁷, Christelle Jouannaud⁷, Marion Fournier⁹, Patrick Soulie¹⁰, Olivier Rigal¹¹, Sylvie Giacchetti¹², Antoine Arnaud¹³, Olivier Arse⁴ne¹⁴, Alexia Savignoni¹⁵, Christelle Mesleard¹⁵, Fabrice André⁵ and Patrick Arveux¹⁶. ¹Institut Curie, Paris, France; ²Gustave Roussy Cancer Campus, Villejuif, France; ³Institut de Cancérologie de Lorraine, Nancy, France; ⁴Institut Curie, Saint-Cloud, France; ⁵Centre Oscar Lambret, Lille, France; ⁶Centre Léon Bérard, Lyon, France; ⁷Centre François Baclesse, Caen, France; ⁸Institut Jean Godinot, Reims, France; ⁹Institut Bergonié, Bordeaux, France; ¹⁰Institut de Cancérologie de l'Ouest, Angers, France; ¹¹Centre Henri Becquerel, Rouen, France; ¹²CHU Saint-Louis, Paris, France; ¹³Institut Sainte Catherine, Avignon, France; ¹⁴CH Blois, Blois, France; ¹⁵R & D Unicancer, Paris, France and ¹⁶Centre Georges François Leclerc, Dijon, France.

Body: Background
There is no large prospective trial assessing mid-term adverse effects of adjuvant chemotherapy. In order to address this question, we developed CANTO (CANCer TOxicities - NCT01993498 - http://etudecanto.org/), a prospective trial dedicated to the quantification of side effects after treatment for patients with early breast cancer and to develop predictors of such toxicities. The aim of this presentation is to assess chemotherapy (CT) practice and to report toxicities that persist 3-6 months after CT.

Methods
CANTO is a prospective study enrolling newly diagnosed invasive cT0-cT3, cN0-3, M0 breast cancer patients (pts) of 26 French comprehensive cancer centers. The study has included 10 500 patients at the time of submission. Pts are assessed at diagnosis, 3-6, 12, 36, 48 and 60 months after treatment completion. CANTO collects >100 items related to toxicities. In the current study, we focus on the first set of data available from the trial (1st database lock, n=6090). We here assess CT practice and toxicities at 3 months.

Results
Information about (neo)adjuvant CT (NACT/ACT) is available in 5805 pts (96%). Median age at diagnosis was 57y (22-93). Pts had HR+/HER2-, HER2+ or triple negative (TN) tumors in 74%, 15% and 11% of cases. Ki67 was assessed in 70%, and genomic tests in 1% of pts, respectively.

Overall, 3074 pts (53%) received CT, either adjuvant (ACT: 76%) or neoadjuvant (NACT: 24%). ACT/NACT pts (84%) received a sequential anthracyclines–taxanes based 6 courses CT schedule. CT was administrated in 44.7%, 87.2% and 92.3% of HR+/HER2+/TN tumors, respectively. ACT was administered in 73.2% of pT2+ pts (vs 36.0% in pT0-1 – p<.001)) and in 74.7% in pN1+ pts (vs 36.7% in pN0 – p<.001)). After NACT, pts had ypT0 (32.3%) and/or ypN0 (64.6%) for an overall 28.9% pCR rate.

We focus here on clinically most relevant patient reported symptoms at 3 m (any grade).

<table>
<thead>
<tr>
<th>side effects at 3m</th>
<th>no CT (%)</th>
<th>CT (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain</td>
<td>76.6</td>
<td>82.1</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Neurological symptom</td>
<td>47</td>
<td>68.7</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>GI symptom</td>
<td>34.3</td>
<td>42.1</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>CV symptom</td>
<td>8.1</td>
<td>10.2</td>
<td>0.011</td>
</tr>
</tbody>
</table>

Pain complaint was recorded in 3596 pts (97.2% of pts with available data), with a median value of 4 on the VAS (range 1-10). In ACT/NACT pts, muscle and joint pain were predominant. Neurological symptoms were seen in 3024 pts (59%), the most frequent pertaining to cognitive disorder (attention trouble, CT: 61.2% vs noCT: 56% - p=.06) and peripheral neuropathy (overall 31%). Paresthesias and sensory neuropathy were much more frequent in CT vs noCT pts: respectively 37.3% vs 20.3% and 25.7% vs 12.8% (both p<.001). Of note, pts receiving paclitaxel had more peripheral neuropathy (92.3% vs 69% in docetaxel pts – p=.07).
Diarrhea was the most frequent GI symptom post CT: 44.5% vs 33.2%, \( p < 0.001 \). CV symptoms (NOS) were slightly more frequent after CT.

**Conclusions**

In this real life, prospective cohort, CT is frequently prescribed and appears in good compliance with current guidelines. Overall, symptoms burden at treatment completion is strikingly high, and much higher in pts receiving CT. A special attention should be given to pain and neurological symptoms. Dedicated questionnaires and sub-studies will explore in depth these side effects. Extended analyses of CT practice and toxicities will be presented.
Body: Background: Nipple-areola tattoos can provide restoration of a natural looking breast that more closely resembles its pre-surgical appearance while avoiding additional surgeries. To date, the majority of nipple-areola tattoo procedures are performed by healthcare providers with minimal training in tattoo procedures. Substandard results explain the high rates of dissatisfaction among women who receive nipple-areola tattoos. In response, professional tattoo artists have emerged as an alternative provider for women seeking reconstruction. However, few studies have examined expectations and experiences of women undergoing nipple-areola tattoo procedures provided by a professional tattoo artist outside of the traditional healthcare setting.

Methods: In-depth interviews were conducted with a racially/ethnically diverse group of 30 women who had undergone nipple-areola tattooing in the past 0-2 years. Interviews were conducted in English, Spanish, Chinese, and Arabic, recorded, and translated and transcribed into English for analysis. A team of three researchers conducted iterative reviews of the data which included closely reading each transcript, coding, running queries of codes, and developing summary documents to highlight recurrent concepts and patterns which were shared and discussed in group meetings.

Results: Interview narratives addressed the often unexpected impact nipple-areola tattooing had on body image, self-esteem, emotional well-being, and interpersonal relationships. Women described their decision-making processes as weighing concern about the needle, the pain, and uncertainty about the tattoo artist, setting for the procedure, and outcome with the opportunity to return to a more “normal” appearance without further surgeries. Women discussed how their initial preconceptions of tattoos and tattoo parlors were ameliorated by the spa-like setting and the tattoo artist's anticipation of such concerns which enhanced her ability to put them at ease and provide professional and compassionate care. Participants noted the integral role the tattoo artist played in their positive experiences, describing her as both an “artist” and “caregiver.” The manner in which she guided them through the decision-making process regarding the color, size, shape, and placement of their nipple-areola tattoo was noted as particularly significant and empowering.

Conclusions: Nipple-areola tattooing is an acceptable and meaningful reconstruction process for medically underserved public hospital patients. Our results indicate that women should be informed of nipple-areola tattooing as an alternative to more invasive, surgical reconstruction options. Results also illustrate how the healthcare system can extend beyond the traditional healthcare setting to include and leverage non-clinical and non-traditional specialists to provide appropriate care and positive breast health outcomes for women. In order to increase access and legitimacy to these services, additional research is needed to understand how to bring tattoo artists “in-house” (i.e., into the medical setting) and how to incorporate tattoo artists into breast health teams.
2017 San Antonio Breast Cancer Symposium

Publication Number: P6-12-20

Title: Impact of symptom burden on employment and quality of life in long-term Mexican breast cancer survivors treated in a tertiary referral center

Yuly A Remolina-Bonilla¹, Eucario León-Rodríguez¹ and Alejandra Armengol-Alonso¹. ¹Instituto Nacional de Ciencias Médicas y Nutrición Dr Salvador Zubirán, Mexico City, Tlalpan, Mexico.

Body: Background: The number of cancer survivors has increased worldwide during the last decade as a result of improvements in the Clinical Oncology Practice. Last year, more than 15.5 million cancer survivors were alive in the United States; 3.5 million were breast cancer survivors (BCS). According to GLOBOCAN 2012, breast cancer incidence in Mexico was 24.8 per 100,000. Studies in Mexican breast cancer patients have reported a five-year overall survival of 80%, expecting 16,000 BCS and 66% of unemployment rates, however, associated factors remain unknown. The aim of this study was to associate symptom burden with employment and quality of life (QOL) in long-term breast cancer survivors (LTBCS).

Patients and methods: A cross-sectional cohort study was performed including 54 patients with history of non-metastatic invasive breast cancer. Inclusion criteria were: ≤ 65 years and ≥ 2 years from diagnosis and off treatment and exclusion criteria was illiteracy. A written questionnaire (Quality of Life-Cancer Survivors, QOL-CS) developed by City of Hope National Medical Center (Spanish version) was used to measure QOL, including 41 items representing four domains: psychological well-being (18 items), physical well-being (8 items), social well-being (8 items), and spiritual well-being (7 items). Items were rated on a scale of 0 (worst outcome) to 10 (best outcome). Information was analyzed using SPSS v.21. A logistic regression was done to predict the impact of the independent variables.

Results: Median age at diagnosis and at the time of the questionnaire application was 50 and 56 years, respectively. The median survival time from diagnosis was 5 years (percentile 25-75 = 3-8.2 years). Stages were: cTNM stage I 18 (33%), stage II 28 (52%), and stage III 8 (15%). Patients received the following treatment: 26 (48%) underwent conservative surgery, 28 (52%) mastectomy; 40 (74%) chemotherapy, 34 (63%) radiotherapy, and 41 (76%) hormonal therapy. Educational attainment was less than college completion in 38 (70%) patients; disease-related work loss in 15 (27.8%), 26 (48.1%) were unemployed, and 18 (33.3%) worked half time. In employed patients the average monthly self-reported income was US $ 300 dollars. A univariate comparison between QOL-CS scores from the National Coalition from Cancer Survivorship (NCCS) and our study found significant differences. Cancer treatment distress (OR 30.5 CI 95% 2.1-437.6 p=0.01) and family distress (OR 16.4 CI 95% 1.0-260.6 p=0.04) were associated with a worst score in the QOL-CS tool. Symptoms such as menstrual change/fertility (OR 1.36 CI 95% 1.04-1.77 p=0.02), physical appearance (OR 1.36 CI 95% 1.01-1.82 p=0.03), and self-concept (OR 1.36 CI 95% 1.00-1.84 p=0.04), were associated with disease-related work loss.

Conclusion: This is one of the first studies that associates symptom burden with employment and QOL in LTBCS in Mexico. Although its limitations (small sample size), the present study highlights the need to carry out specific research to investigate the local needs in order to design public policies to subsequently improve the impact on QOL and employment in these patients in Latin America.
Title: Breast cancer survivors undergoing survivorship visits at Johns Hopkins are a high-risk population

Body: **Background:** Survivorship care plans (SCPs) are strongly recommended for all breast cancer survivors to address sequelae of cancer care, plan cancer surveillance and screening, and encourage health promotion and care coordination. Ongoing studies are evaluating the impact of SCPs in cancer survivor populations and the role of survivorship visits (SVs) as an intervention. Here we describe characteristics and outcomes of patients who participated in SVs at Johns Hopkins (JH).

**Methods:** We retrospectively reviewed the charts of patients who participated in a SV with one of two nurse practitioners ~1-3 months after completion of locoregional therapy and initial systemic therapy, as referred by their JH breast cancer provider. We collected patient demographics, comorbidity status, tumor characteristics, treatments received, and responses to GAD7 (generalized anxiety disorder 7-item), PHQ9 (patient health questionnaire-9), and a symptom questionnaire. Characteristics of SV participants were compared to analytical breast cancer cases from the JH Cancer Registry (JHCR 2010-2015), matched for stage.

**Results:** 87 women (stages I-III) who participated in a SV in 2010-2016 were identified. Compared to patients in the JHCR (n=2,942), the SV cohort was younger (age ≤50, 43% v 34%, p=0.14), more likely to be African American (33% v 22%, p=0.04), and more likely to have a higher TNM stage (I, 26% v 49%; II, 48% v 37%; III, 25% v 15%, p<0.001), node-positive status (60% v 33%, p<0.001), hormone receptor-negative disease (44% v 18%, p<0.001), and HER2-positive disease (38% v 14%, p<0.001). The SV cohort was also more likely to receive chemotherapy (94% v 43%, p<0.001) and undergo radiation therapy (78% v 54%, p<0.001). The SV cohort had a higher recurrence event rate than the JHCR cohort (11.5% v 8.0%) and a shorter median follow-up (886 v 1292 days), suggestive of a higher risk profile. In the SV cohort, a comparison of comorbidities at breast cancer diagnosis versus time of SV visit identified a significant increase in the prevalence of peripheral neuropathy (9% v 73%, p<.001), anemia (15% v 50%, p<.001), lymphedema (0% v 28%, p<.001), anxiety (15% v 38%, p<.001), and depression (13% v 29%, p<.001). Patients in the SV cohort were overweight at diagnosis (body mass index, median 29 [IQR 24, 32]). At the time of the SV, patients reported symptoms of sleep difficulty (53%), numbness or tingling (46%), weight changes (45%), muscle aches (44%), and pain (37%).

**Conclusions:** Patients who participated in SVs had high-risk cancers and, compared to baseline, a higher frequency of comorbidities that are potentially associated with breast cancer and its treatment. These data can inform future breast cancer survivorship care models as they describe a population that may be at greater risk for worse cancer and non-cancer outcomes, and that might benefit more from interventions like SCPs and SVs. Ongoing studies are identifying optimal target populations, appropriate timing of such interventions, and informative measures of patient-centered outcomes.

**Funding:** Komen Maryland/Komen Scholar SAC110053 (ACW).
**Title:** Sexual health in long-term breast cancer survivors

Sara V Soldera¹, Marguerite Ennis², Ana E Lohmann³ and Pamela J Goodwin³. ¹Princess Margaret Cancer Centre, University of Toronto, Toronto, ON, Canada; ²Applied Statistician, Markham, ON, Canada and ³Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, University of Toronto, Toronto, ON, Canada.

**Body:**

**Background** Significant sexual dysfunction is reported in women with breast cancer (BC) in the years following diagnosis. It is unclear whether symptoms persist over time in BC survivors (BCS) as long-term data compared to healthy controls is lacking. We compared sexual functioning in long-term BCS to controls and explored the impact of adjuvant therapy on sexual health.

**Methods** A cohort of women with localized BC recruited from 1989 to 1996 was prospectively followed as previously described. BCS without recurrence and controls without BC were contacted between 2005 and 2007 and answered self-reported quality of life questionnaires. Sexual health was measured with the Sexual Activity Questionnaire (SAQ). Vasomotor, gynecological and bladder symptoms were scored using the Menopausal Symptom Scale (scale ranges 0-4) based on the Breast Cancer Prevention Trial Symptom Checklist. Regression analysis was used to compare groups, with and without adjustment for age (quadratic) and menopausal status. P values <0.05 were considered significant.

**Results** 248 of 285 BCS and 159 of 167 controls completed the SAQ. The median time from diagnosis of BCS was 12.5 years. BCS were slightly older (61.9 vs 59.1 years, p=0.0004) and somewhat more likely to be post-menopausal (94.4 vs 85.5%, p=0.0025) than controls. Overall, fewer BCS were sexually active than controls (45.2 vs 59.7%, p=0.0041). This difference was no longer significant when adjusted for age and menopausal status (odds ratio 0.68, p=0.075). In those sexually active, no significant differences were noted on the SAQ Pleasure and Discomfort scales. Differences in adjuvant treatment were not significantly associated with being sexually active or the SAQ subscales. BCS scored higher (worse) on the gynecological and bladder symptom scale than controls (0.66 vs 0.43, p=0.0036, adjusted difference 0.24, p=0.0029; 0.60 vs 0.41, p=0.02, adjusted difference 0.18, p=0.029 respectively), but no difference was seen in vasomotor scores. Gynecological symptom scores were greatest in BCS who received adjuvant chemotherapy.

**Conclusion** Despite more frequent long-term gynecological and bladder symptoms, sexual health is similar in BCS and controls. Adjuvant chemotherapy is associated with persistent gynecological symptoms and interventions aimed at improving these could improve quality of life.
**Pharmacotherapeutic adherence of adjuvant hormone therapy in patients with breast cancer at the National Cancer Institute of Mexico**


**National Cancer Institute Mexico, Mexico City, Mexico.**

**Body: Background:**
Pharmaceutical care is related to the use of medications; In the past decade, the development of new oral drugs for breast cancer has increased. The adherence rate for oral medications is often suboptimal, decreasing survival rates, increasing the risk of recurrence, and increasing costs in health care.

**Methods:** We analyzed a total of 1179 patients with breast cancer in stage 0 - IIIC, during the period from April 2016 to April 2017, who received adjuvant hormonal treatment. The percentage of adherence per clinical stage was evaluated, through a survey based on 6 questions, non adherence to the treatment was defined, if the patients answered affirmatively to 2 questions of detachment. We also examined the use of alternative therapies, polypharmacy and the use of different hormonal oral therapies.

**Results:** A total of 1179 breast cancer patients who received adjuvant hormone treatment were surveyed. Four hormonal drugs were examined, of which 56.8% corresponded to Tamoxifen, 18.75% to Letrozole, 13.04% to Exemestane and 11.42% to Anastrozole.

It lists the total number of patients per clinical stage and the percentage of adherence they had: Stage 0, 65 patients (73%), Stage IA 284 patients (69.7%), Stage IB 6 patients (50%), stage IIA 316 patients (73.7%), stage IIB 231 patients (71.4%), stage IIIA 172 patients (70%), stage IIIB 73 patients (63%), and stage IIIC 32 patients (68%) respectively. It was found that 3% of the patients were also using alternative therapies (herbal products and dietary supplements). And 12% of patients with polypharmacy were found. The main cause of non-adherence was the forgetting of medication intake.

**Conclusions:** The results showed similar percentages of adherence to the hormonal treatment in the different clinical stages. The use of alternative therapies and polypharmacy did not influence adherence to treatment. Of the different hormonal drugs, 56.8% corresponded to the use of Tamoxifen; No differences were found in the adhesion by hormonal drug received or by toxicity to it.

At present, continuous communication between health professionals and patients, as well as education about the disease and possible toxicity to treatment, to improve adherence to hormonal drugs is important.
Body: Background The risk of psoriasis in breast cancer patients is largely unknown, as available evidence is limited to case findings. We systematically examined the incidence and risk factors of psoriasis in breast cancer patients.

Methods Using Cox regression models, a Swedish nationwide cohort of 56,235 breast cancer patients (2001-2012) was compared to 280,854 age-matched reference individuals from the general population to estimate the incidence and hazard ratio (HR) of psoriasis. We also calculated HRs for psoriasis according to treatment, genetic and lifestyle factors in a regional cohort of 8,987 patients.

Results In the nationwide cohort, 599 breast cancer patients were diagnosed with psoriasis during 307,684 person-years (median: 5.1 years) compared to 2,795 cases in the matched individuals during 1,666,038 person-years. Breast cancer patients were at an increased risk of psoriasis (HR=1.17; 95% CI=1.07-1.28) and its most common subtype (psoriasis vulgaris; HR=1.33; 95% CI =1.17-1.52). The risk of psoriasis vulgaris was long-term increased up to 12 years after diagnosis. Treatment specific analyses indicated an increased risk of psoriasis in patients treated with radiotherapy (HR=2.44; 95% CI=1.44-4.12) and mastectomy (HR=1.54, 95% CI=1.03-2.31). Apart from treatment-specific effects, we identified genetic predisposition, obesity and smoking as independent risk factors for psoriasis in breast cancer patients.

Table 1. Hazard ratios for psoriasis according to treatment characteristics

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total No.</th>
<th>No. of cases</th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endocrine therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1533</td>
<td>27</td>
<td>1.00 (REF)</td>
<td>1.00 (REF)</td>
</tr>
<tr>
<td>Yes</td>
<td>7100</td>
<td>121</td>
<td>0.90 (0.59-1.36)</td>
<td>0.80 (0.52-1.24)</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>5544</td>
<td>102</td>
<td>1.00 (REF)</td>
<td>1.00 (REF)</td>
</tr>
<tr>
<td>Yes</td>
<td>3070</td>
<td>46</td>
<td>0.82 (0.57-1.19)</td>
<td>0.70 (0.47-1.04)</td>
</tr>
<tr>
<td>Radiotherapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>2061</td>
<td>23</td>
<td>1.00 (REF)</td>
<td>1.00 (REF)</td>
</tr>
<tr>
<td>Yes</td>
<td>6574</td>
<td>125</td>
<td>1.78 (1.14-2.78)</td>
<td>2.44 (1.44-4.12)</td>
</tr>
<tr>
<td>Surgery</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumpectomy</td>
<td>5203</td>
<td>94</td>
<td>1.00 (REF)</td>
<td>1.00 (REF)</td>
</tr>
<tr>
<td>Mastectomy</td>
<td>3459</td>
<td>55</td>
<td>0.96 (0.69-1.34)</td>
<td>1.54 (1.03-2.31)</td>
</tr>
</tbody>
</table>

Model 1: adjusted for age and calendar period of breast cancer diagnosis. Model 2: model 1 plus all the treatment factors.

Table 2. Hazard ratios of psoriasis according to genetic and lifestyle factors

<table>
<thead>
<tr>
<th></th>
<th>Total No.</th>
<th>No. of cases</th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Model 1: adjusted for age and calendar period of breast cancer diagnosis. Model 2: model 1 plus all the treatment factors.
<table>
<thead>
<tr>
<th>PRS score</th>
<th>1440</th>
<th>13</th>
<th>1442</th>
<th>36</th>
<th>1483</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRS score Tertile 1</td>
<td>1.00 (REF)</td>
<td>1.00 (REF)</td>
<td>2.74 (1.45-5.17)</td>
<td>2.83 (1.50-5.34)</td>
<td>2.94 (1.57-5.49)</td>
<td>2.98 (1.59-5.58)</td>
</tr>
<tr>
<td>BMI 25-30 kg/m²</td>
<td>2331</td>
<td>40</td>
<td>1.00 (REF)</td>
<td>1.00 (REF)</td>
<td>1.18 (0.73-1.92)</td>
<td>1.15 (0.71-1.87)</td>
</tr>
<tr>
<td>&gt;30 kg/m²</td>
<td>536</td>
<td>19</td>
<td>2.29 (1.32-3.98)</td>
<td>2.10 (1.20-3.68)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical activity per week</td>
<td>0 hours</td>
<td>21</td>
<td>1.00 (REF)</td>
<td>1.00 (REF)</td>
<td>0.77 (0.45-1.33)</td>
<td>0.77 (0.44-1.32)</td>
</tr>
<tr>
<td>&gt;2 hours</td>
<td>1910</td>
<td>30</td>
<td>0.56 (0.32-0.98)</td>
<td>0.59 (0.33-1.03)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regular smoker</td>
<td>No</td>
<td>26</td>
<td>1.00 (REF)</td>
<td>1.00 (REF)</td>
<td>1.65 (1.04-2.61)</td>
<td>1.59 (1.00-2.52)</td>
</tr>
</tbody>
</table>

Genetic predisposition for psoriasis was defined by a polygenic risk score (PRS) including 35 SNPs for psoriasis susceptibility. Model 1: adjusted for age and calendar period of breast cancer diagnosis. Model 2: all of the risk factors were put into the model, including treatment.

**Conclusions** The incidence of psoriasis is elevated among breast cancer patients, with treatment, lifestyle and genetic factors defining the individual risk profile. Our findings underline the complex etiology of psoriasis in breast cancer patients and may help to assess individual risk of psoriasis after a breast cancer diagnosis.
2017 San Antonio Breast Cancer Symposium

Title: Sexual function in breast cancer survivors stratified by adjuvant therapies and surgical modalities

Christy Gandhi¹, Elizabeth C Butler¹, Sarah Pesek², Rebecca Kwait³, Melissa Clark⁵, Christina Raker⁴, Ashley Stuckey¹ and Jennifer Gass¹. ¹The Program in Women's Oncology, Women and Infants Hospital, Warren Alpert Medical School of Brown University, Providence, RI; ²St. Peter's Health Partners, Albany, NY; ³Exeter Hospital, Exeter, NH; ⁴The Division of Research Women and Infants Hospital, Warren Alpert Medical School of Brown University, Providence, RI and ⁵Center for Health Policy and Research, University of Massachusetts Medical School, Shrewsbury, MA.

Body: Background: Breast cancer survivors comprise the largest group of cancer survivors in the United States. With increasing duration of recurrence-free survival, more women sustain the long-term consequences of treatment affecting quality of life. Our study aims to investigate associations of surgical modality, chemotherapy, radiation and endocrine therapy on sexual function of breast cancer survivors.

Methods: An anonymous, cross-sectional survey of 585 patients in surveillance after breast cancer therapy was conducted at a single academic breast cancer center. The survey questions included surgical modality, adjuvant therapies, and sexual function using the Female Sexual Function Index (FSFI). Median FSFI (mFSFI) results were stratified by surgery type and by receipt of chemotherapy, radiation, and endocrine therapy. Statistical analysis was performed using Fisher's exact and the Wilcoxon rank-sum tests or the Kruskal-Wallis test.

Results: Of the 585 respondents, 55.3% were < 60 years old and 51.3% were sexually active. Surgical modalities included lumpectomy (L=406), mastectomy with reconstruction (MR=129) and mastectomy without reconstruction (M=50). For adjuvant therapy, 405 patients received radiation, 276 received chemotherapy, 117 reported tamoxifen (TAM) use, and 189 reported aromatase inhibitor (AI) use.

For all patients stratifying for receipt of adjuvant chemotherapy or radiation therapy, there was no difference in mFSFI scores (radiation: 26.7 vs 28 p=0.2, chemo: 26.5 vs. 27.5 p=0.1). Regarding endocrine therapy, AI patients had statistically significant lower mFSFI compared to no endocrine therapy or TAM (22.2 vs. 27.9 vs 29.6, p <= .0001).

mFSFI Scores by Adjuvant Therapies

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>mFSFI</th>
<th>Range</th>
<th>IQR</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiation: Yes</td>
<td>207</td>
<td>26.7</td>
<td>7.2-36.0</td>
<td>21.5-30.5</td>
<td></td>
</tr>
<tr>
<td>Radiation: No</td>
<td>71</td>
<td>28.0</td>
<td>10.7-34.8</td>
<td>22.4-31.7</td>
<td>0.2</td>
</tr>
<tr>
<td>Chemo: Yes</td>
<td>133</td>
<td>26.5</td>
<td>7.2-36.0</td>
<td>21.0-30.5</td>
<td></td>
</tr>
<tr>
<td>Chemo: No</td>
<td>145</td>
<td>27.5</td>
<td>10.8-35.7</td>
<td>22.2-31.2</td>
<td>0.1</td>
</tr>
<tr>
<td>TAM and AI</td>
<td>2</td>
<td>23.1</td>
<td>21.5-24.6</td>
<td>21.5-24.6</td>
<td>0.2</td>
</tr>
<tr>
<td>TAM Only</td>
<td>74</td>
<td>29.6</td>
<td>11.6-35.7</td>
<td>24.6-32.3</td>
<td>0.07</td>
</tr>
<tr>
<td>AI Only</td>
<td>85</td>
<td>22.2</td>
<td>7.2-35.7</td>
<td>18.1-28.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Neither</td>
<td>112</td>
<td>27.9</td>
<td>9.8-36.0</td>
<td>23.2-31.0</td>
<td>Ref</td>
</tr>
</tbody>
</table>

Patients on AI had significantly lower scores in all FSFI domains except for orgasm. For patients on TAM, the desire and lubrication domains showed statistically significantly lower scores compared to nonusers of endocrine therapy (p= 0.03). There was no significant difference in mFSFI by surgical type, although patients with MR had the highest score at 28.5. Controlling for surgical modality and stratifying by adjuvant treatment, radiation and chemotherapy did not have statistically
significant effects on sexual function. Patients reporting L and TAM use had mFSFI significantly higher than those with AI use (29.4 vs 23.7, p=0.005). For MR patients reporting AI use, mFSFI was significantly lower than those on no endocrine therapy (18.1 vs 29.6, p<0.0001).

Conclusions: Our study does not show an association of surgical modality, receipt of chemotherapy or radiotherapy and sexual function as measured by FSFI. Patients receiving endocrine therapy with an AI had significantly lower sexual function scores, than those who received no endocrine therapy or those on tamoxifen. These data may guide clinicians in counseling sexually active breast cancer patients in surgical planning and in survivorship.
Title: The axonal damage marker, serum phosphorylated neurofilament heavy subunit, as a potential marker of chemotherapy-induced neuropathy

Kumiko Kida¹, Masahiko Sumitani², Toru Ogata³, Rina Kotake¹, Akina Natori⁴, Jun Hashimoto¹, Toshio Shimokawa², Hideko Yamauchi¹ and Teruo Yamauchi¹. ¹St.Luke’s International Hospital, Tokyo, Japan; ²University of Tokyo, Tokyo, Japan; ³National Rehabilitation Center for Persons with Disabilities, Saitama, Japan; ⁴Princess Margaret Cancer Centre, Tronto, Canada and ⁵Wakayama Medical University, Wakayama, Japan.

Body: Background: Chemotherapy-induced neurologic disorders such as peripheral neuropathy and cognitive disturbance are clinically significant problems for cancer survivors, but their objective assessment methods have not been established. We previously reported in a cross-sectional study that the serum phosphorylated neurofilament heavy subunit (pNF-H), a biomarker of axonal damage, was increased in breast cancer patients treated with chemotherapy. The aim of this study is to temporally assess the neurological adverse events and evaluate the association of serum pNF-H level with cognitive functions and neuropathy following sequential chemotherapy.

Methods: Thirty-five breast cancer patients who received neoadjuvant or adjuvant chemotherapy were enrolled prospectively. They underwent brain MRI and cognitive function tests including Controlled Oral Word Association (COWA), Trail Making Test (TMT), and Hopkins Verbal Learning Test-Revised (HVLT-R) before chemotherapy (baseline), one month after completing sequential chemotherapy (post-phase) and more than six months after completing chemotherapy (late-phase). Serum pNF-H levels and questionnaires reporting peripheral neuropathy were measured at the three phases, and every 3 weeks during chemotherapy. Brain MRI volumetry was calculated by the automatic analysis software, BAAD® (Brain Anatomical Analysis using Dartel). The correlations among cognitive functions, brain volume, peripheral neuropathy and serum pNF-H levels were statistically analyzed.

Results: Patients' median age was 48 years (range 24-71). A decrease of more than 10% in cognitive function test (COWA) scores was seen in 10 cases (31%) at post-phase. A brain volume loss of more than 10% was seen in 5 cases (15%) at post-phase. The correlation between brain volume change and cognitive disturbance was not significant (p=0.45) and both changes were improved at late-phase. A peripheral neuropathy grade above CTCAE grade 2 was seen in 19 cases (54%). The neuropathy was significantly more severe in anthracycline followed by taxane regimen than taxane followed by anthracycline during chemotherapy (p=0.016), although this difference was not seen at the late-phase (p=0.08). An elevated serum pNF-H level at baseline was seen in only one case, and this case demonstrated the cognitive disturbance, brain volume loss, and peripheral neuropathy following chemotherapy. During chemotherapy, pNF-H was elevated in 24 patients (69%), with especially higher levels noted during the taxane regimen compared to the anthracycline regimen (p=0.019). In the cases treated with anthracycline followed by taxane, the taxane-phase elevation was especially significant (p=0.014). The maximum pNF-H level during taxane therapy was significantly correlated with peripheral neuropathy grade (p=0.002). At late-phase, the significant reduction of pNF-H level was seen in all cases.

Conclusions: Change of cognitive function, brain volume and peripheral neuropathy was observed following chemotherapy in breast cancer patients. This study suggests that the serum axonal damage marker, pNF-H, may reflect chemotherapy-induced neuropathy.
2017 San Antonio Breast Cancer Symposium

Publication Number: P6-13-02

Title: Increased mortality risk among elderly patients with early stage triple negative breast cancer who did not receive adjuvant or neoadjuvant therapy

Sacha Satram-Hoang¹, Preeti S Bajaj², Alisha Stein², Khang Q Hoang¹, Faiyaz Momin¹, Patricia Cortazar² and Carolina Reyes². ¹Q.D. Research, Granite Bay, CA and ²Genentech, Inc., South San Francisco, CA.

Body: Background: Worldwide, breast cancer (BC) is the most frequently diagnosed cancer and the leading cause of cancer death in women. The triple negative (TN) subtype accounts for up to 20% of BC and has a poorer prognosis compared to other subtypes. We set out to evaluate treatment patterns and survival associated with receiving adjuvant or neo-adjuvant therapy in an older, demographically diverse population of patients with TNBC.

Methods: The analysis included 10,694 patients with first primary TNBC from the linked SEER-Medicare database. Patients were diagnosed with Stage I-III disease between January 1, 2001-December 31, 2011, ≥66 years, continuously enrolled in Medicare Parts A and B in the year prior to diagnosis, and underwent breast cancer surgery within 6 months after diagnosis. Unadjusted Kaplan Meier analyses and time-varying Cox proportional hazards regression adjusting for patient characteristics assessed overall survival. Date of last follow-up was December 31, 2013.

Results: There were 4,807 (45%) patients treated with adjuvant/neo-adjuvant chemotherapy and 5,887 (55%) untreated. Treatment rates increased over the study time-period from 45% in 2001 to 52% in 2011 (p<.0001). Compared to treated patients, untreated patients were older (78 vs. 73 years), had earlier stage disease (57% vs. 31% Stage I), lower tumor grade (34% vs. 23% grade 1/2), smaller tumors (57 vs.40% <2cm), poorer performance (13% vs. 6%), higher comorbidity burden (45% vs. 37% NCI Comorbidity Score ≥ 1) and were less likely to receive radiotherapy (47% vs. 65%; p<0.0001). The median unadjusted overall survival was 94.3 months for the overall population and was longer for treated patients (101.5 months) compared to untreated patients (88.4 months; log rank p <.0001). In the adjusted Cox model, there was a 28% higher risk of death in untreated compared to treated patients (HR=1.28; 95% CI=1.19-1.38). The model also showed that as age, stage, tumor size, tumor grade, and comorbidity score increased, mortality risk also significantly increased. Having poor performance indicators was also significantly associated with higher mortality risks, while prior radiotherapy was associated with lower risks.

Conclusions: Although therapy use has increased over time, this large observational study confirmed that 55% of elderly patients with Stage I-III TNBC are not receiving adjuvant/neo-adjuvant therapy following diagnosis. As a result, untreated patients exhibited a significantly elevated risk of death compared to those who received treatment. The results of this study highlight the unmet need in this patient population and provide an important context to optimize disease management in real-world settings.
2017 San Antonio Breast Cancer Symposium

Publication Number: P6-13-03

Title: Breast cancer-specific mortality (BCSM) in patients with node-positive (N+) breast cancer (BC) treated based on the 21-gene assay in clinical practice

Steven Shak¹, Debbie McCullough¹ and Valentina I Petkov². ¹Genomic Health, Inc., Redwood City, CA and ²National Cancer Institute, Bethesda, MD.

Body: Introduction: The Recurrence Score® (RS) assay was shown in SWOG 8814 to predict chemotherapy (CT) benefit for patients (pts) with N+ BC and RS ≥31 but not RS <18. As we await the randomized RxPONDER results for RS 0-25, we characterized BCSM for RS groups (cutoffs of 11, 18, 25, and 31) in the large population-based SEER study of pts treated based on RS results.

Methods: RS results were provided electronically to SEER registries per their linkage methods (Petkov npj Breast Cancer 2016). Eligible pts were diagnosed (Jan 2004 - Dec 2012) with N+ (micrometastases, 1-3 positive nodes), HR+, HER2-negative BC, and had no prior malignancy or multiple tumors. BCSM estimates by reported CT use were computed using standard cutpoints of 18 and 31 and TAILORx/RxPONDER cutoffs of 11 and 25, and should be interpreted cautiously given known under-reporting of CT use to SEER and lack of randomization.

Results: Among 6,483 pts with RS results, 1,312 (20%) had RS <11, 2,478 (38%) had RS 11-17, 1,831 (28%) had RS 18-25, 432 (7%) had RS 26-30, and 430 (7%) had RS ≥31. There was a significant association between RS results and BCSM (p<0.001) without and with adjustment for age, tumor size, and grade. Reported CT use and 5-y BCSM increased with increasing RS result (Table). For pts with RS <11 and RS 11-17, CT use was reported in approximately a quarter of pts, and 5-y BCSM was low regardless of CT use. For pts with RS 18-25, CT use was more common and the 5-y BCSM was about 2%. For pts with RS of 26-30 or ≥31, CT was common, and higher 5-y BCSM was observed.

5-y BCSM, by RS Group and Reported CT Use

<table>
<thead>
<tr>
<th>RS group</th>
<th>CT reported as 'No/Unknown'</th>
<th>5-y BCSM (95% CI)</th>
<th>CT reported as 'Yes'</th>
<th>5-y BCSM (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;11</td>
<td>1066 (81%)</td>
<td>1.8% (0.7%, 4.6%)</td>
<td>246 (19%)</td>
<td>1.3% (0.3%, 5.3%)</td>
</tr>
<tr>
<td>11-17</td>
<td>1869 (75%)</td>
<td>0.5% (0.2%, 1.1%)</td>
<td>609 (25%)</td>
<td>2.3% (0.9%, 5.8%)</td>
</tr>
<tr>
<td>18-25</td>
<td>1034 (56%)</td>
<td>2.0% (1.0%, 3.9%)</td>
<td>797 (44%)</td>
<td>1.9% (0.8%, 4.5%)</td>
</tr>
<tr>
<td>26-30</td>
<td>144 (33%)</td>
<td>7.7% (2.8%, 20.3%)</td>
<td>288 (67%)</td>
<td>4.0% (1.6%, 10.1%)</td>
</tr>
<tr>
<td>≥31</td>
<td>99 (23%)</td>
<td>11.9% (5.3%, 25.6%)</td>
<td>331 (77%)</td>
<td>11.1% (6.9%, 17.6%)</td>
</tr>
</tbody>
</table>

Conclusion: Reported CT use and 5-y BCSM in N+ BC increased with increasing RS results in “real-world” clinical practice. 5-y BCSM with RS <18 was less than 2% in pts with no or unknown CT use. 5-y BCSM in pts treated based on RS results appears to increase considerably with RS >25.
Title: IMPACt trial: MammaPrint and BluePrint molecular subtyping guide treatment decisions in breast cancer

Hatem Soliman¹, Esther Rehmus², Varsha Shah³, Gordan Srkalovic⁴, Reshma Mahtani⁵, Ellis Levine⁶, Blanche Mavromatis⁷, Jayanthi Srinivasiah⁸, Mohamad Kassar⁹, Robert Gabordi¹⁰, Erin Yoder¹², Rubina Qamar¹¹, William Audeh¹² and IMPACt Investigators Group¹³. ¹Moffitt Cancer Center, Tampa, FL; ²Akron General Medical Center, Akron, OH; ³Columbia St. Mary's Hospital, Milwaukee, WI; ⁴Sparrow Cancer Center, Lansing, MI; ⁵University of Miami, Miami, FL; ⁶Roswell Park Cancer Institute, Buffalo, NY; ⁷Western Maryland Health System, Cumberland, MD; ⁸Dekalb Medical Center, Decatur, GA; ⁹Community Hospital Northwest Oncology, Munster, IN; ¹⁰St. Joseph's Women's Hospital, Tampa, FL; ¹¹Aurora Health Care, Milwaukee, WI; ¹²Agendia, Irvine, CA and ¹³IMPACt Investigators Group.

Background: IMPACt is a prospective, case-only study to measure the effect of MammaPrint (MP) and BluePrint (BP) on treatment decisions in breast cancer patients. Here, we report the results of the primary objective in women aged ≥18 years with histologically proven invasive stage I-II, hormone receptor (HR) positive, and HER2-negative breast cancer.

Methods: The study included 369 women from 18 US institutions. The recommended treatment plan was captured before and after receiving results for MP and BP. Treatment was started after obtaining results. In addition to the effect of results on physician treatment decisions involving chemotherapy (CT) and physician confidence, the distribution of MP High Risk (HR) and Low Risk (LR) patients was also evaluated.

Results: MP classified patients to 62% (n=228) LR and 38% (n=141) HR. Treatment decisions were changed for 25% (n=92) of women after receiving MP and BP results. Of the LR patients initially prescribed CT, 68% (45/66) had CT removed from their treatment recommendation. Of the HR patients who initially were not prescribed CT, 66% (42/64) had CT added. Overall, 89% (202/228) of LR patients did not receive CT, and likewise 84% (119/141) of HR patients did receive CT after receiving MP. Among those who did not change treatment (n=277), 68% of physicians reported having greater confidence in their prescribed therapy.

Conclusions: The IMPACt trial shows MP generates a 25% overall treatment change in clinical practice. The highest impact is for women with LR results, where 68% are spared chemotherapy in favor of endocrine therapy alone. Additionally, 73% of physicians report having higher confidence in treatment decisions for their patient after MP.

Table 1: Treatment changes

<table>
<thead>
<tr>
<th>Treatment Decision Pre- to Post-MP</th>
<th>MP HR</th>
<th>MP LR</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT to CT</td>
<td>77</td>
<td>21</td>
<td>98</td>
</tr>
<tr>
<td>no CT tp CT</td>
<td>42</td>
<td>5</td>
<td>47</td>
</tr>
<tr>
<td>CT to no CT</td>
<td>0</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>no CT to no CT</td>
<td>22</td>
<td>157</td>
<td>179</td>
</tr>
<tr>
<td>Total</td>
<td>141</td>
<td>228</td>
<td>369</td>
</tr>
</tbody>
</table>
Body: Introduction
Chemotherapy (CT) is the standard of care for most triple negative breast cancer (TNBC). Chemotherapy is less commonly recommended in older than younger patients. We aim to explore the frequency of CT delivered in elderly patients when compared to young patients.

Methods
Patients ≤50yrs and ≥70 yrs with stage I-III TNBC defined as ER <10% PR < 10% HER2 IHC < 3+ or FISH < 2.0 and treated at our institution from 2000-2011 were identified from our institutional breast cancer database. Clinicopathologic features were retrieved and co-morbidity indexes (CI) were calculated. Patients were grouped by age and CT use, and features were compared between groups using chi-square tests. Cause of death was reported as dead of disease (DOD) or dead of other causes (DOC) when available; otherwise, it was recorded as dead of unknown causes (DUC). OS survival was estimated using the Kaplan-Meier (KM) methods. Cumulative incidence functions for competing risks were calculated and compared between groups using Gray's test. Competing risks regression was performed for multivariate analysis.

Results
We identified 901 pts with TNBC; 664 (73.7%) were ≤50yrs and 237 (26.3%) were ≥70 yrs. Median followup is 7 yrs (range, 0-16.8yrs).
Younger women diagnosed with TNBC were more likely to have stronger family history of breast cancer (p<.001), to present with palpable masses (p<.001), higher nuclear grade (NG) (p<.001), larger tumors (p=.04), more involved nodes (p=.01), advanced tumor stage (p=.02) and to receive systemic chemotherapy (<.001). Anthracycline-based chemotherapy was administered to 486 (80.3%) in women ≤50yrs and only to 42 (36.5%) in the ≥70 yrs cohort (p<0.001). Chemotherapy data was missing on 2 pts in ≤50 yrs and 4 pts ≥70 for a total cohort of 662 patients ≤50 yrs and 233 pts ≥70 yrs.
The 5 year rates of DOD were similar between both groups at 10.6% (range, 8.3-13.2) for pts ≤50yrs and 10.8% (range, 7.0-15.4) (p=0.52) for the older group; meanwhile, the 5 year OS rates were significantly different between both groups at 87.5% (range, 84.7-90.0) for pts ≤50yrs and 74.3% (range, 68.2-80.0) (p<0.001) for the older group since older women die at higher rates from causes other than disease.
CT was given to 115 (49%) patients of the ≥70 yrs cohort with a selection biased by larger tumors (p<.001) and more advanced stages (p<.001). There was no significant difference however, between tumor size (p=0.47) and stage (p=0.98) when comparing the 609 (92%) pts ≤50 yrs and the 115 (49%) of ≥70 yrs patients who received CT.
When categorized based on age and receipt of CT, in the 662 pts ≤ 50 yrs, 609 (92%) and 53 (8%) received CT vs no CT respectively; in the 233 pts ≥70 yrs, 115 (49%) and 118 (50%) received CT vs no CT; the cumulative incidence curves for DOD were not statistically different for the four groups (p=0.85) at 5 years.

Conclusions
In our series, CT was given to 92% of patients ≤ 50 yrs of age. In the elderly pts ≥ 70 yrs of age, CT was limited to 50% of patients, namely those with worse clinicopathologic features.
Body: Background: Despite an increase in prevalence of breast cancer in elderly patients, studies often exclude this population. As a result, there is a lack of evidence regarding treatment options and outcomes in the elderly. The 70-gene signature (70-GS) MammaPrint (MP) provides risk of recurrence for early-stage breast cancer (ESBC) patients, utilizing tumor biology, and developed independent of clinico-pathological factors (CPF). Tests utilizing CPF such as immunohistochemistry (IHC) assess a tumor by looking at cell surface characteristics which do not fully indicate whether chemotherapy (CT) or endocrine treatment (ET) is sufficient for response. Here we report a subpopulation from the COmmunity based retrosPective study comparing the 70-GS with common clinical-Pathological criteria in selecting patients for adjuvant chemotherapy in breast cancER with 0-3 positive nodes (COPPER).

Methods: 517 early stage breast cancer cases diagnosed and classified as low risk or high risk by MP were identified (2009-2016). 246 of these patients were ≥65 years old with an average age at diagnosis of 70, ages ranging from 65-98. Genomic risk was determined by the 70-GS. ER, HER2, nodal status, tumor grade and tumor size were utilized to develop a model to categorize all patients as either clinically-low risk (CLR) or high risk (CHR) per these standard CPF.

Results: 40% (51/129) of these older patients who appeared CHR with ESBC were genomically low risk according to the 70-GS and therefore would be at a reduced risk of recurrence without adjuvant treatment. Of 123 LR 70-GS patients, 90 (73%) did omit CT in alignment with their LR MP result, 2 (2.2%) had events (deaths due to metastasis). Similarly, 38% (45/117) of patients who appeared CLR were genomically high risk by the 70-GS and would likely recur without additional adjuvant treatment. Of 123 HR MP patients, the majority, 78 (63%) received CT in agreement with their MP result. There were 5 events in this group.

Conclusions: Elderly patients were safely spared or assigned treatment based on a 70-GS result. These data highlight the importance of considering tumor biology in treatment decisions versus clinico-pathological methods alone.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>70-GS HR</th>
<th></th>
<th>70-GS LR</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Count All</td>
<td>Count Events</td>
<td>% Events</td>
<td>Count All</td>
</tr>
<tr>
<td>Chemo (with or without ET)</td>
<td>78</td>
<td>5</td>
<td>6.4%</td>
<td>20</td>
</tr>
<tr>
<td>No Chemo (ET Only or Untreated)</td>
<td>34</td>
<td>2</td>
<td>5.9%</td>
<td>90</td>
</tr>
<tr>
<td>Unknown</td>
<td>11</td>
<td>0</td>
<td>0.0%</td>
<td>13</td>
</tr>
<tr>
<td>All</td>
<td>123</td>
<td>7</td>
<td>5.7%</td>
<td>123</td>
</tr>
</tbody>
</table>
Chemotherapy with and without trastuzumab or no treatment in elderly patients with HER2 amplified breast cancer at a single center

Shirin Muhsen\textsuperscript{1}, Chau Dang\textsuperscript{2}, George Plitas\textsuperscript{1}, Kenneth Seier\textsuperscript{3}, Michelle Stempel\textsuperscript{1}, Sujata Patil\textsuperscript{3}, Monica Morrow\textsuperscript{1} and Mahmoud El-Tamer\textsuperscript{1}. \textsuperscript{1}Memorial Sloan Kettering Cancer Center; \textsuperscript{2}Memorial Sloan Kettering Cancer Center and \textsuperscript{3}Memorial Sloan Kettering Cancer Center.

Body: Introduction
Trastuzumab with systemic chemotherapy has shown an improvement in outcomes for patients (pts) with HER2 amplified/overexpressed (HER2+) breast cancer. Pts enrolled onto trials were young with a minority of pts at $\geq$65 years (yrs) of age. Herein, we report the administration of systemic treatment (ST) (chemotherapy and/or trastuzumab) verus no treatment in elderly pts at a single center.

Methods
Patients $\geq$65 yrs with stage I-III HER2+ (defined as IHC 3+ or FISH $>$2.0) breast cancer, treated at Memorial Sloan Kettering Cancer Center between 2000-2012, were retrospectively identified from our database. Clinicopathologic features were retrieved and co-morbidity indexes (CI) were calculated. Pts were divided by hormone receptor (HR) (defined as ER $>$10% and/or PR $>$10%) status into HER2+HR- and HER2+HR+. Each group was further divided by use of ST into: chemotherapy and trastuzumab (CT+T), chemotherapy alone (CT) or no systemic treatment (No Rx). Patients receiving neoadjuvant ST or trastuzumab only as ST were excluded from the KM analysis. Primary objective was to identify patterns of treatment recommendation in the elderly population. We explored disease-free survival (DFS) as estimated using the Kaplan-Meier (KM) method.

Results
We identified 300 pts $\geq$65 yrs with HER2+ tumors. 128 (42.7%) were HER2+HR- and 172 (57.3%) were HER2+HR+. The median follow-up for all patients was 6.1 years (range, 0.07-16.7). In the HER2+HR- group, 63 (49.2%) patients received CT+T, 25 (19.5%) CT alone, and 40 (31.3%) had no Rx. Anthracycline based chemotherapy was administered to 57/88 (65%) of patients on CT. Women receiving chemotherapy with or without trastuzumab were younger (65-70 vs $>$70 years of age) (p=.002) and had more advanced tumor stages (p=.003). Their respective 5-yr DFS KM estimates were 0.84, 0.80, and 0.61 (logrank p=0.06).
In the HER2+HR+ group, 77 (44.8%) patients received CT+T, 22 (12.8%) CT alone, and 73 (42.2%) had no Rx. Anthracycline based chemotherapy was administered to 51/99 (51%) of patients on CT. Endocrine therapy was given to 153/172 (89%) of the total cohort. Women receiving chemotherapy with or without trastuzumab were younger (p$<$.001), and had higher nuclear grade (NG) (p=.04), more lymphovascular invasion ($<$,.001) and more advanced tumor stages (p=.002). Their respective 5-yr DFS KM estimates were 0.84, 1.00, and 0.83 (log rank p=0.02).

Conclusions
At a single center, in the elderly populations at $\geq$65 years of age with HER2+ HR- and HER2+HR+ breast cancer, pts who received systemic treatment were younger and had higher stage of disease than those who received no treatment. In an exploratory analysis, there appeared to be a benefit of systemic treatment in pts in the HER+HR- group.
Title: A novel comparative analysis approach to personalize chemotherapy dose in early breast cancer

Melissa D Perri1, Sandeep Singhal1, Kathleen Hegadoren1, Colleen Norris1, John Mackey1, Ian Paterson1 and Edith Pituskin1.

1University of Alberta, Edmonton, AB, Canada.

Body: Background: Worldwide, body surface area [BSA] is used to calculate chemotherapy dose. The BSA formula was originally developed in 1916, derived from height and weight, with no consideration of other patient characteristics. Most chemotherapy agents have a narrow therapeutic index and are distributed in lean body mass [LBM], leading to under- or over-dosing and deleterious effects to major organs when body composition is not considered. To date, while experts worldwide acknowledge the limitations and risks of BSA dosing, no practical approach to personalizing chemotherapy dose has been developed. Ideally, body composition would be assessed by tests already routinely performed, avoiding unnecessary radiation exposure, clinic visits, discomfort to the patient, and cost. The majority of patients undergo cardiac imaging prior to chemotherapy. We hypothesized that clinical parameters routinely performed prior to chemotherapy could predict LBM in early breast cancer patients.

Method: Early stage breast cancer patients (n = 45) enrolled in the Multidisciplinary Team Intervention in Cardio-Oncology (TITAN) study underwent pre-treatment cardiac MRI, body composition (iDEXA) and laboratory (complete blood cell count and chemistry). Cardiac MRI and iDEXA are considered ‘gold standard’ imaging modalities, the accuracy of which allow for significantly reduced sample size.

Our modeling approach, which is novel in this area, aimed to select the best combination of parameters with the most predictive ability of total lean mass (iDEXA). The parameters included in study are: cardiac MRI metrics (LV mass, cardiac output), and laboratory parameters associated with major organ function (albumin, creatinine, bilirubin). All parameters were tested using univariate, multivariate and subset selection approach. Akaike's Information Criterion (AIC) was used to measure model quality, with lower AIC values indicating closer prediction.

Results: The univariate analysis of each parameter independently showed LV mass is most predictive with AIC 857.8, while combination of all parameter in multivariate fashion show improvement in prediction with AIC 851. The subset selection approach shows, Adjusted R2 with 4 parameters had AIC 849.14, Schwartz’s information criterion (BIC) with 2 parameters had AIC 849.66 and Mallows' C Selection (Cp) model with 3 parameters had the least AIC 848.71 value (P < 0.001).

Conclusion: Our comparative analysis showed that the Cp model with 3 parameters (LV mass, cardiac output and bilirubin) has high prediction ability of LBM. This model will form the basis of a personalized formula for chemotherapy dose calculation. We expect this work to result in optimal cancer-specific outcomes while reducing short and long-term toxicities associated with necessary chemotherapy.
2017 San Antonio Breast Cancer Symposium

Publication Number: P6-14-01

**Title:** The effect trial: A randomized phase II trial evaluating two different doses of weekly (W) NAB-paclitaxel (NP) as first-line chemotherapy in older breast cancer (BC) patients (pts)

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**Body:** Background: W taxanes (T) are commonly used in the treatment of older BC pts, with neurotoxicity (NTX) and fatigue being dose-limiting toxicities with a possible negative impact on function. No prospective data exists on the safety and efficacy of W NP in this population. NP might be of particular value in older pts, due to no need for premedication with steroids and shorter time to recovery from neurotoxicity than conventional T, resulting in a reduced risk of exacerbation of comorbidities such as hypertension and diabetes, and possibly of functional decline (FD). Methods: Pts aged ≥ 65 years (y) with Her-2 negative or Her-2 positive (+), but contraindicated to anti-Her-2 therapy, advanced BC were randomized to receive NP as first-line chemotherapy at either 100 (Arm A) or 125 mg/m² (Arm B), days 1, 8, 15 q 28. The primary end-point was event-free survival (EFS). An event was either disease progression (PD), death, or FD - defined as a decrease of at least 1 point from baseline values of activities of daily living (ADL) or instrumental ADL (IADL), deemed by the investigator as treatment-related and confirmed at the subsequent cycle. Secondary endpoints included progression-free survival (PFS), response rate (RR) in pts with measurable disease, and incidence of adverse events (AEs). Results: From January 2013 to September 2016, 160 pts were randomized in 15 Italian centres; all but 2 who never started NP were eligible for final analysis. Pts median age was 72y (range 65-84) in Arm A and 73y (range 65-88) in Arm B. Median ECOG performance status was 0 (range 0-2). Baseline IADL impairment was reported in 20 pts (25%) in both arms. >80% pts had ER+ tumors; 2 pts had HER2+ disease. Visceral disease was present in 71% (Arm A) and 70% (Arm B) of pts. Prior exposure to T in the neo/adjuvant setting was 14% (Arm A) and 13% (Arm B). Median number of delivered cycles of NP was 6 (range 1-28 in Arm A, and 1-22 in Arm B), with 3 pts still on treatment. Dose reductions were similarly reported (72% of pts Arm A, 78% of pts Arm B). At a median follow-up of 21 months (mos) (Interquartile range 14-28.4) 140 events were observed. Arm A/Arm B: PD n=53(67%)/n=52(66%); death n=3(4%)n=5(6%). Outcomes data are reported in the following table:

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Arm A</th>
<th>Arm B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median EFS, mos (90% CI)</td>
<td>6.2 (5.5-8.4)</td>
<td>6.4 (5.8-7.7)</td>
</tr>
<tr>
<td>Median PFS, mos (95% CI)</td>
<td>8.3 (5.9-10.5)</td>
<td>8.8 (7.4-10.3)</td>
</tr>
<tr>
<td>RR (95% CI)</td>
<td>37% (25-50)</td>
<td>42% (30-54)</td>
</tr>
</tbody>
</table>
Fatigue (Arm A: grade (G)2 29%, G3 11%; Arm B: G2 46%, G3 5%) and NTX (Arm A: G2 15%, G3 4%; Arm B: G2 28%, G3 8%) were the most frequently reported AEs. No G4 AEs were reported with the exception of neutropenia (1 pt in arm A) and leucopenia (3 pts in Arm A, 1 pt in arm B). 1 G5 (sepsis) was recorded in Arm B. NTX was reported as the reason for treatment discontinuation in 21 pts (13%) of whom 16 (21%) in arm B. Conclusion: Looking at classical study endpoints (PFS, RR), both doses of NP are active in older pts. However, 17% of pts had to stop treatment due to FD, assessed according to predefined criteria. Due to similar efficacy and reduced NTX, W NP 100 is the suggested dose to be used in older pts with advanced BC.
Title: Real-life activity of eribulin among metastatic breast cancer patients in the multicenter national observational ESME program

William Jacot¹, Pierre-Etienne Heudel², Julien Fraisse¹, Sophie Gourgou¹, Séverine Guiu¹, Florence Dalenc³, Barbara Pistilli⁴, Mario Camponè⁵, Christelle Levy⁶, Marc Debled⁷, Marianne Leheurteur⁸, Marie Chaix⁹, Claudia Lefevre¹⁰, Anthony Goncalves¹¹, Lionel Uwer¹², Jean-Marc Ferrero¹³, Jean-Christophe Eymard¹⁴, Thierry Petit¹⁵, Marie-Ange Mouret-Reynier¹⁶, Coralie Courtinard¹⁷, Paul Cottu¹⁸, Mathieu Robain¹⁷ and Audrey Mailliez¹⁹. ¹Institut du Cancer de Montpellier (ICM) Val d’Aurelle, Montpellier, France; ²Centre Léon Bérard, Lyon, France; ³IUCT Oncopole, Toulouse, France; ⁴Gustave Roussy, Villejuif, France; ⁵Institut de Cancérologie de l’Ouest, Saint-Herblain, France; ⁶Centre François-Baclesse, Caen, France; ⁷Institut Bergonié, Bordeaux, France; ⁸Centre Henri Becquerel, Rouen, France; ⁹Centre Georges Francois Leclerc, Dijon, France; ¹⁰Centre Eugène Marquis, Rennes, France; ¹¹Institut Paoli-Calmettes, Marseille, France; ¹²Institut de Cancérologie de Lorraine, Vandœuvrière-lès-Nancy, France; ¹³Centre Antoine Lacassagne, Nice, France; ¹⁴Institut de Cancérologie Jean Godinot, Reims, France; ¹⁵Centre Paul Strauss, Strasbourg, France; ¹⁶Centre Jean-Perrin, Clermont-Ferrand, France; ¹⁷UNICANCER, Paris, France; ¹⁸Institut Curie, Paris, France and ¹⁹Centre Oscar Lambret, Lille, France.

Body: Background: In 2014, UNICANCER (composed of 18 French Comprehensive Cancer Centers) launched the Epidemiological Strategy and Medical Economics (ESME) program to investigate real-world data in solid tumors. Real-world data give the opportunity to assess for the activity of specific drugs outside clinical trials. Eribulin is approved for pre-treated metastatic breast cancer (MBC). Marketing authorization has been granted in France in July 2012. However few data are available regarding its efficacy in real life. We evaluated eribulin use as second and third line of chemotherapy in MBC patients from the ESME database.

Methods: Data from all newly diagnosed MBC patients having initiated at least one treatment between Jan. 2008 and Dec. 2014 are included in the ESME database. Data were collected retrospectively using a clinical trial-like methodology. Primary endpoint was overall survival (OS), defined from the starting date of second or third line chemotherapy (eribulin versus other chemotherapy). Progression-free survival (PFS) was calculated as a secondary endpoint.

Results: Of 16,703 MBC patients included in the ESME database, 7,412 received at least 2 lines of chemotherapy: eribulin/other chemotherapy, total 1,966/5,446, second line 363/5,446, third line 654/2,669. Depending on second or third line chemotherapy use classification, median age was 59 years (range 20-97) and 58 year (range 21 – 94), triple negative tumors accounted for 20% and 19% of cases, and median follow-up reached 26 months and 22 months respectively.

<table>
<thead>
<tr>
<th></th>
<th>OS eribulin (months)</th>
<th>OS other chemotherapy (months)</th>
<th>p</th>
<th>PFS Eribulin (months)</th>
<th>PFS other chemotherapy (months)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Second</td>
<td>12.4 (11.3-15.1)</td>
<td>11.8 (11.3-12.3)</td>
<td>0.465</td>
<td>4.1 (3.7-4.9)</td>
<td>4.1 (4.0-4.3)</td>
<td>0.9225</td>
</tr>
<tr>
<td>line</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Third</td>
<td>10.3 (9.3-11.5)</td>
<td>7.7 (7.3-8.0)</td>
<td>&lt;.0001</td>
<td>3.6 (3.2-3.9)</td>
<td>3.0 (2.9-3.2)</td>
<td>0.0058</td>
</tr>
</tbody>
</table>

Supportive analyses (using a propensity score for adjustment and as a matching factor for nested case–control analyses) and sensitivity analyses will be available for full presentation at the meeting.

Conclusion: In this large-scale real-life setting, MBC patients treated with third line eribulin showed an improved OS and PFS compared with those receiving another chemotherapy. The difference was not statistically significant for second line treatment.
Title: Randomized phase II study evaluating weekly oral vinorelbine versus weekly paclitaxel in estrogen receptor positive, HER2-negative patients with advanced breast cancer (NorBreast-231 trial)

Matti Aapro¹, Manuel Ruiz Borrego², Elzbieta Staroslawksa³, Serafin Morales⁴, Saverio Cinieri⁵, Ruffo De Freitas Junior⁶, Laura Garcia Estevez⁷, Eva Szombara⁸, Helene Hervieu⁹, Melanie Groc⁹, Gustavo Villanova⁹ and Roberto Hegg¹⁰. ¹Breast Center, Genolier Cancer Center, Genolier, Switzerland; ²Hospital Virgen del Rocio, Seville, Spain; ³Oncology Center, Lublin, Poland; ⁴Hospital Arnau de Vilanova, Lleida, Spain; ⁵Ospedale A. Perrino, Brindisi, Italy; ⁶Hospital Universitario Jorge Araujo, Goiania, Brazil; ⁷Hospital San Chinarro, Madrid, Spain; ⁸Marie Curie Oncology Center, Warsaw, Poland; ⁹Pierre Fabre Médicament, Boulogne-Billancourt, France and ¹⁰Hospital Perola Byington, Sao Paulo, Brazil.

Body: Rationale: Quality of Life is of prime importance in Advanced Breast Cancer (ABC), a mostly non-curable disease. Both paclitaxel (P) and vinorelbine as single-agent chemotherapy (CT) are recommended treatment options in the management of ABC non-responsive to hormone therapy (HT) and with no visceral crisis. These agents are active with a good tolerance profile. The benefits and safety of oral vinorelbine (OV) and weekly P have however never been evaluated in a face to face trial.

Methods: Main eligibility criteria included: age ≥18 years, documented locally recurrent or metastatic involvement previously untreated by CT, estrogen receptor positive disease previously treated by at least one HT, HER2-negative status, Karnofsky PS ≥70 and presence of at least one measurable lesion. Study treatment (until progression): Arm A, OV 80 mg/m² weekly (following a first cycle at 60 mg/m² and dose escalation to 80 in the absence of grade 3 or 4 toxicity); Arm B: paclitaxel 80 mg/m² weekly. One cycle was defined as three weeks of treatment. Patients were stratified according to prior taxane (yes/no) and visceral metastases (yes/no). Primary endpoint was disease control rate (DCR), defined as confirmed complete response/partial response/stable disease of a minimum duration of 6 weeks.

Results: 131 pts have been randomized (OV 66; P 65). Baseline patient characteristics (Arms A/B): median age 58/61 years; median number of previous HT 2/2; prior (neo)adjuvant CT 74/72%; prior anthracycline 64/62%; prior taxane 39/42%; ≥3 metastatic sites 42/48%; visceral metastases 80/78%. Safety: the most common non-haematological related G3/4 adverse events (≥3% patients) were: fatigue 7.6/1.5%, peripheral neuropathy 0/4.6%, nausea 3/0%, diarrhoea 3/1.5%, vomiting 3/0%, constipation 3/1.5%; alopecia (G2) was present in 1.5/33.8%; no toxic deaths observed. Efficacy: DCR in the intent-to-treat population was [95%CI] 75.8 [63.6-85.5]/75.4 [63.1-85.2] %; overall response was 20/40%; stable disease was 56/35% respectively. Treatment exposure, other efficacy / safety parameters and quality of life results will be presented during the meeting.

Conclusion: Both OV and P reached similar DCR rates of 75%. As expected, each regimen presented a specific tolerance profile, with, in particular, a lower incidence of alopecia and peripheral neuropathy with OV.
Title: Is fat mass more effective than body mass index (BMI) to predict toxicity in early breast cancer patients treated with doxorubicin and cyclophosphamide?

Muhammet Ali Kaplan1, Hemrin Kavak2, Zuhat Urakci1, Necip Nas2, Zeynep Oruç1, Halis Yerlikaya1, Nadiye Akdeniz1 and Abdurrahman Isikdogan1. 1Dicle University and 2Dicle University.

Body: Patients and Methods: We performed a retrospective cohort study of 207 operated breast cancer women treated with doxorubicin (60 mg/m^2) and cyclophosphamide (600 mg/m^2) for adjuvant setting between 2007 and 2016. Patients’ demographic features, toxicities, fat mass, body mass index (BMI) and body surface area (BSA) were evaluated in their charts. Patients were evaluated according to fat mass (high ≥35% vs. low <35%), BMI (obese ≥30 kg/m^2 vs. nonobese <30 kg/m^2), and BSA (≥1.73 vs. <1.73) levels.

Results: Median age was 46 (23-82) and 61.8% of the patients (n=128) were premenopausal. Median fat mass, BSA, and BMI level was 37 (10-55), 1.75 (1.3-2.27) and 29.4 (16-54.7), respectively. Grade ≥3 toxicity was observed in 68 patients (%32.9). Although, in univariate analyses [table 1] there are no differences in terms of grade ≥3 toxicity according to BMI (in obese and nonobese patients, 34.8% vs. 31.4%, respectively, p=0.589), and BSA (in patients with m^2 ≥1.73 vs. m^2 <1.73, 35.8% vs. 29.6%, respectively, p=0.344), statistically significant difference was observed according to fat mass (fat mass high and low, 39.1% vs. 25.0%, respectively, p=0.031). In multivariate analyses [table 2], fat mass [<35 vs. >35, OR (odds ratio): 2.341 %95CI:0.39-5.27, p=0.040] was affect grade ≥3 toxicity, while BMI [<30 vs. >30; OR:0.876 %95 CI=0.392-1.959, p=0.748], BSA [<1,73 vs. >1.73 m^2, OR:0.956, %95CI:0.450-2.034, p=0.908], age [<50 vs. >50, OR:2.171, %95CI:0.338-13.956, P= 0.414], menopausal status [premenopausal vs. postmenopausal, OR:4.374, %95CI:0.661-28.964, p=0,126], stage [1,2 vs 3, OR:0.535, %95CI:0.279-1.024, p=0.059] and histologic subtype [ductal vs. others; OR:2.010 %95 CI=0.368-2.010, p=0.729] was not.

Grade ≥3 toxicity according to BMI, BSA, and fat mass

<table>
<thead>
<tr>
<th>Method</th>
<th>Grade ≥3 toxicity</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (obese vs nonobese)</td>
<td>34.8% vs. 31.4%</td>
<td>0.589</td>
</tr>
<tr>
<td>BSA (≥1.73 vs. m^2 &lt;1.73)</td>
<td>35.8% vs. 29.6%</td>
<td>0.344</td>
</tr>
<tr>
<td>Fat mass (≥35% vs. &lt;35%)</td>
<td>39.1% vs. 25.0%</td>
<td>0.031</td>
</tr>
</tbody>
</table>

BMI: Body mass index, BSA: body surface area

Multivariate analyses for grade ≥ 3 toxicity

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Odds Ratio</th>
<th>%95 Confidence Interval</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat mass (≥35% vs. 35%)</td>
<td>2.341</td>
<td>0.39-5.27</td>
<td>0.040</td>
</tr>
<tr>
<td>BMI (≥30 vs. &lt; 30)</td>
<td>0.876</td>
<td>0.392-1.959</td>
<td>0.748</td>
</tr>
<tr>
<td>BSA (&lt;1,73 vs. &gt;1.73)</td>
<td>0.956</td>
<td>0.450-2.034</td>
<td>0.908</td>
</tr>
<tr>
<td>Age (≥50 vs. &lt;50)</td>
<td>2.171</td>
<td>0.338-13.956</td>
<td>0.414</td>
</tr>
<tr>
<td>Menopausal status</td>
<td>4.374</td>
<td>0.661-28.964</td>
<td>0.126</td>
</tr>
<tr>
<td>Stage</td>
<td>0.535</td>
<td>0.279-1.024</td>
<td>0.059</td>
</tr>
<tr>
<td>Histologic type</td>
<td>2.010</td>
<td>0.368-2.010</td>
<td>0.729</td>
</tr>
</tbody>
</table>

BMI: Body mass index, BSA: Body surface area
Discussion: BSA-based dosing has been widely adopted in oncology as a means of safely administering cytotoxic drugs. In our study demonstrated that fat mass is most valuable than BSA and BMI for evaluation to grade ≥3 toxicity for breast cancer patients treated with AC in the adjuvant setting.
2017 San Antonio Breast Cancer Symposium

Publication Number: P6-14-05

Title: Phase 2 study evaluating the efficacy and safety of eribulin mesylate administered biweekly for patients with human epidermal growth factor receptor 2-negative metastatic breast cancer

John Smith II,1,2 Amy Irwin2,3, Lori Jensen2,4, Karen Tedesco2,5, Soamnauth Misir6, Wei Zhu6, Ana Almonte6, Yaohua He6, Martin Olivo6 and Joyce O'Shaughnessy2,7. 1Compass Oncology, Portland, OR; 2US Oncology, The Woodlands, TX; 3Virginia Cancer Specialists, Leesburg, VA; 4Rocky Mountain Cancer Centers, Boulder, CO; 5New York Oncology Hematology, Albany, NY; 6Eisai Inc., Woodcliff Lake, NJ and 7Baylor University Medical Center, Texas Oncology, Dallas, TX.

Body: Background: Eribulin mesylate, a microtubule inhibitor, is approved in the US for the treatment of patients (pts) with metastatic breast cancer (MBC) who have previously received at least 2 chemotherapeutic regimens for the treatment of metastatic disease, including an anthracycline and a taxane. The recommended dose is 1.4 mg/m² (equivalent to 1.23 mg/m² eribulin [expressed as free base]) on day (D) 1 and D8 of a 21-D cycle. However, this schedule can result in dose delays and reductions due to myelosuppression. A dosing regimen of eribulin (1.4 mg/m²) administered intravenously (IV) biweekly (Q2W; on D1 and D15) in 28-D cycles was evaluated with the intent of improving eribulin's safety profile without compromising efficacy.

Methods: Female pts with human epidermal growth factor receptor (HER)2-negative MBC, who had received 2-5 prior chemotherapy regimens and had ECOG PS ≤2 were enrolled in 12 sites in the US. Prophylactic granulocyte colony-stimulating factor (G-CSF) was not allowed. If neutropenia occurred, growth factors were used during eribulin treatment at the physician's discretion. Primary endpoints were objective response rate (ORR) and disease control rate (DCR). Secondary endpoints were progression-free survival (PFS), overall survival (OS), dose intensity (measured by feasibility rate), safety and tolerability.

Results: Median age of the 58 enrolled pts was 64 yrs (range: 38-85). The majority of pts (93%) had ECOG PS ≤1, and 12% of pts had triple-negative MBC. Number of prior chemotherapeutic regimens: 2 (17% of patients), 3 (24%), 4 (27%), and 5 (31%). 76% Of pts had visceral disease and 86% had previous taxane therapy. ORR (95% confidence interval [CI]) was 12% (5-24), DCR (CR+PR+SD) was 65% (95% CI: 51-77), and CBR (CR+PR+SD ≥23 weeks) was 30% (95% CI: 18-43) [n=57]. Median PFS (95% CI) was 3.6 mo (2.9-4.1). Median OS (95% CI) was 13.2 mo (10.6-not estimable). 6-Month and 12-month OS rates were 84% and 54%, respectively. Dose intensity measured by the feasibility rate (defined as the percentage of pts completing the first 2 and 4 cycles without a dose delay >5 days or dose reduction due to an adverse event [AE]) was 70% and 46%, respectively. The most frequent AEs (all grades) were neutropenia (69%), fatigue (48%), alopecia (45%), and constipation (36%). 22% Of pts had grade (G) 1 alopecia and 22% of pts had G2 alopecia. 72% Of pts had G3/4 AEs: neutropenia, 57%, and peripheral neuropathy, 12%. G3 peripheral sensory neuropathy occurred in 9% of pts, with no G4 incidence. There were 2 deaths (1 sepsis, 1 acute respiratory failure), which were considered not related to treatment. 50% (29/58) Of all patients received at least 1 dose of growth factor and 70% (28/40) of patients with neutropenia received growth-factor support.

Conclusions: Tumor response rates and OS of this treatment schedule in a heavily pretreated patient population were similar compared to previously reported phase 3 studies of eribulin. The toxicities associated with biweekly eribulin were manageable.
Oral etoposide (VP-16) in heavily pre-treated metastatic breast cancer: A multicenter national observational study

Florence Lerebours¹, Matthieu Carton¹, Joy Bacrie¹, Jean-Yves Pierga¹, Roman Rouzier¹, Mahasti Saghatchian², Florence Dalenc³, Audrey Mailliez⁴, Christelle Levy⁵, Nelly Firmin⁶, Marc Debled⁷, Marianne Leheurteur⁸, Isabelle Desmoulins⁹, Claudia Lefevre⁴, Anthony Goncalves¹¹, Lionel Uwer¹⁵, Jean-Marc Ferrero¹³, Jean-Christophe Eymard¹⁴, Thierry Petit¹⁵, Marie-Ange Mouret-Reynier¹⁶, Irwin Piot¹⁸, Genevieve Perrocheau¹⁷, Christian Caillot¹⁸ and David Perol¹⁹. ¹Institut Curie, Saint-Cloud, France; ²Gustave Roussy, Villejuif, France; ³IUCT, Toulouse, France; ⁴Centre Oscar Lambret, Lille, France; ⁵Centre Francois Baclesse, Caen, France; ⁶ICM, Montpellier, France; ⁷Institut Bergonie, Bordeaux, France; ⁸Centre Henri Becquerel, Rouen, France; ⁹Centre Georges-Francois Leclerc, Dijon, France; ¹⁰Centre Eugene Marquis, Rennes, France; ¹¹Institut Paoli-Calmettes, Marseille, France; ¹²ICL, Vandoeuvre-les-Nancy, France; ¹³Centre Antoine Lacassagne, Nice, France; ¹⁴Institut Jean Godinot, Reims, France; ¹⁵Centre Paul Strauss, Strasbourg, France; ¹⁶Centre Jean Perrin, Clermont-Ferrand, France; ¹⁷ICO, Nantes, France; ¹⁸Unicancer, Paris, France and ¹⁹Leon Berard.

Body: Background

20-30% of breast cancer (BC) patients will develop distant metastases. Despite important improvement achieved in the management of metastatic breast cancer (MBC), median overall survival (OS) still ranges from 14 to 50 months, depending on tumor subtypes. Patients often receive many successive lines of therapy over the course of their disease. Few data on efficacy of 3rd and subsequent lines of chemotherapy are available. Although oral etoposide has been used for years in heavily pre-treated MBC patients, real-life data supporting its efficacy are lacking.

Methods

The primary objective was to assess progression free survival (PFS) in MBC patients having started oral etoposide as 3rd line chemotherapy or more, using the French Epidemiological Strategy and Medical Economics (ESME) database, established in 2014 by Unicancer. Secondary objectives were overall survival (OS) and descriptive and prognostic analyses.

Results

Of 16703 MBC patients included in the ESME program and covering the 2008-2014 period, 323 received at least 14 days of oral etoposide. Amongst them, 5 were men, 46 received etoposide as 1st or 2nd line chemotherapy, and 7 had no information available regarding line of treatment, leaving 265 for analysis. All received oral etoposide, 255 as monotherapy while 10 pts combined with trastuzumab for HER2+ MBC. Patients' and tumour characteristics are shown in table 1.

The mean line of chemotherapy with oral etoposide was 5 (range 3-12). With a median follow-up of 32.2 months [CI95%: 22.0-42.1], median PFS and OS (from initiation of oral etoposide) were 3.1 months [95% CI 2.8-3.7] and 7.4 months [95% CI 6.0-9.9], respectively.

No predictive factor for PFS could be identified (age at MBC diagnosis, BC subtype, number of metastatic sites, presence of visceral metastases, disease-free interval, line of treatment). Only a trend for longer PFS was seen in patients > 50 years old, with longer disease-free interval and receiving etoposide earlier. Triple-negative MBC had shorter OS compared to other subtypes (p=0.03). Of note, OS was stable around 7 months for subsequent lines following a 3rd line of chemotherapy.

<table>
<thead>
<tr>
<th>Age at MBC diagnosis</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 50 yo</td>
<td>83</td>
<td>31.3</td>
</tr>
<tr>
<td>≥ 50 yo</td>
<td>182</td>
<td>68.7</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Primary BC subtype</th>
<th>n</th>
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</thead>
<tbody>
<tr>
<td>ER+ HER2-</td>
<td>166</td>
<td>62.6</td>
</tr>
<tr>
<td>HER2+</td>
<td>38</td>
<td>14.3</td>
</tr>
<tr>
<td>Triple negative</td>
<td>56</td>
<td>21.1</td>
</tr>
<tr>
<td>ND</td>
<td>5</td>
<td>1.9</td>
</tr>
<tr>
<td>----</td>
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<td>----</td>
</tr>
<tr>
<td><strong>Number of metastatic sites</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>154</td>
<td>58.1</td>
</tr>
<tr>
<td>≥ 2</td>
<td>111</td>
<td>41.9</td>
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<td><strong>Visceral metastasis</strong></td>
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<tr>
<td>No</td>
<td>132</td>
<td>49.8</td>
</tr>
<tr>
<td>Yes</td>
<td>133</td>
<td>50.2</td>
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<tr>
<td><strong>Disease-free interval</strong></td>
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<tr>
<td>de novo</td>
<td>40</td>
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<tr>
<td>6-24 months</td>
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<tr>
<td>24-60 months</td>
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<td>27.9</td>
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<tr>
<td>&gt; 60 months</td>
<td>110</td>
<td>41.5</td>
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<tr>
<td><strong>Chemotherapy line</strong></td>
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<tr>
<td>3</td>
<td>63</td>
<td>23.8</td>
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<td>47</td>
<td>17.7</td>
</tr>
<tr>
<td>6</td>
<td>43</td>
<td>16.3</td>
</tr>
<tr>
<td>≥ 7</td>
<td>47</td>
<td>17.7</td>
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</tbody>
</table>

**Conclusions**

Oral etoposide may achieve significant disease control in heavily pre-treated MBC, with a 3.1-month median PFS and independently from prognostic factors. Moreover, etoposide has the major advantage of an oral drug administered at home with a low cost. A case-control study is ongoing.
**Title:** Outcome after neoadjuvant chemotherapy in elderly breast cancer patients – a pooled analysis of individual patient data from eight prospectively randomized controlled trials

Gabriel von Waldenfels¹, Sibylle Loibl², Jenny Furlanetto³, Machleidt Anna⁴, Bianca Lederer⁵, Carsten Denkert¹, Claus Hanusch⁶, Jens Huober⁷, Christian Jackisch⁸, Sherko Kümmel⁹, Gunter von Minckwitz², Andreas Schneeweiss⁴, Michael Untch⁹, Kerstin Rhiem⁸, Peter A Fasching¹⁰ and Jens U Blohmer¹.¹ Charité University Hospital, Berlin, Germany; ²German Breast Group, Neu-Isenburg, Hessen, Germany; ³University Hospital Cologne, Cologne, Nordrhein-Westfalen, Germany; ⁴Rotkreuzklinikum Munich, Munich, Bayern, Germany; ⁵University Hospital Ulm, Ulm, Baden-Wuerttemberg, Germany; ⁶Sana Klinikum Offenbach, Offenbach, Hessen, Germany; ⁷Kliniken Essen Mitte, Essen, Nordrhein-Westfalen, Germany; ⁸University Hospital Heidelberg, Heidelberg, Baden-Wuerttemberg, Germany; ⁹Helios Klinikum Berlin Buch, Berlin, Germany and ¹⁰University Hospital Erlangen, Erlangen, Bayern, Germany.

**Body:**

**Introduction:** Recent studies showed the high and independent impact of age (<40 years) on pathologic complete remission (pCR) and prognosis for patients undergoing neoadjuvant chemotherapy (NACT). Some physicians might not consider elderly patients (>65 years) for NACT due to poor prognosis or higher toxicity. The aim of this analysis is to help selecting appropriately elderly women who would benefit from NACT. Secondly, survival parameters will be investigated in several clinical and histological subgroups.

**Methods:** From 1998 to 2010, eight prospectively randomized German Breast Group (GBG) trials of anthracycline- and taxane-based NACT were performed and analyzed in this study.

**Results:** Compared to the overall average, women older than 65 years had significant larger tumors and more overall lymph node involvement. Also, compared to patients younger than 51 years, they had more lobular invasive tumors. Histologically, they had more G2 tumors, more estrogen-receptor positive tumors. PCR (ypT0 ypN0) was strongly associated with age: >65y: 11.7%; 51-65y: 14.1%; 40-50y: 17.3%; <40y: 20.9%. The multivariable logistic regression analysis of clinical parameters showed that young age, clinical stage T4, invasive ductal cancer and poor differentiated breast cancer are predictive for high pCR. The multivariate analyses of molecular subgroups also showed that age >65years is a predictor of significant (p<0.05) lower pCR in TNBC and HR positive/HER2- breast cancers. Nonetheless, in this cohort, HER2+ patients showed pCR rates as high - and for HR+/HER2+ even higher - pCR rates compared to younger patients.

**Discussion:** This study underlines the unfavorable impact of higher age on pCR, but it shows nevertheless a realistic chance for pCR if NACT is applied - especially for HER2+ patients. Furthermore, elderly patients in this analysis with non-TNBC have a good prognosis (comparable to younger patients) regarding OS, even if they do not have pCR.
Body: Background: The magnitude of tumor infiltrating lymphocytes (TIL) is a predictor of good prognosis in the context of neoadjuvant chemotherapy (NAC) in breast cancer, and is expressed in the capacity to achieve a pathological complete response (pCR) and therefore to obtain a major survival. The characteristics of TIL are influenced by different clinical and pathological features as well as its predictive value. The goal of this study is to define the different associations between level of stromal (sTIL) and interstitial (iTIL) tumor infiltrating lymphocytes and pathological and clinical feature of breast cancer.

Methods: The level of TIL was analyzed in 483 patients diagnosed with breast cancer of the Instituto Nacional de Enfermedades Neoplasicas from 2003 to 2014. We evaluate patient characteristics such as age, clinical stage, histological subtype and grade, clinical T, N and M, presence of estrogen (ER) and progesterone (PgR) receptors, HER-2 expression and molecular subtype as well as pathological response. Results: We found a positive association between sTIL and iTIL proportion and histological grade (G1-2 vs G3, p<0.05 and p<0.05 respectively), hormone receptor (ER- vs ER+PgR- vs ER+PgR+, p<0.05 and p=0.001 respectively), molecular subtype (Luminal A vs Luminal B vs HER2 vs Triple Negative (TN), p<0.05 and p=0.002 respectively), clinical N (positive vs negative, p=0.006 and p<0.05 respectively). Also we found association between sTIL with lymph nodes after surgery (negative vs positive, p=0.028) and pathological response (pCR vs <pCR, p=0.018), there was no association between sTIL and iTIL with age, histological subtype, clinical T and M. Conclusions: The level of tumor infiltrating lymphocytes are affected by different clinical and pathological features, they are present in both lobular and ductal breast cancer without significant difference; higher level of TIL are associated with high histological grade, ER-, TNBC and high clinical N to diagnosis; and specially sTIL are associated with to achieve a negative lymph node after NAC and obtain a pathological complete response.
Title: Randomized controlled trial of neoadjuvant eribulin mesylate versus paclitaxel in women with operable breast cancer (JONIE-3 study)

Body: Background:
Although treatment of eribulin mesylate (E) improved overall survival in metastatic breast cancer (BC) patients, little is known about the efficacy in early BC. The hypothesis of this study is that sequential administration of E followed by FEC would have less toxic, particularly peripheral neuropathy, and also have similar effect compared to paclitaxel (P) followed by FEC as primary systemic therapy (PST) for woman with operable BC.

Methods:
This is a phase II multicenter open label study (UMIN000012817). Patients (pts) were randomly assigned to either E (1.4mg/m2, d1 and d8, q21 days, 4 cycles) + FEC (fluorouracil 500 mg/m2, epirubicin 100 mg/m2, and cyclophosphamide 500 mg/m2) or P (80mg/m2, weekly, 12 cycles) + FEC as PST. HER2+ patients were allowed to receive trastuzumab. Stratification factors were ER, HER2, and menopausal status. Primary endpoint was the incidence of peripheral sensory and motor neuropathy (PSN and PMN) with Grade 1 or higher according to CTCAE ver.4.0. Secondary endpoints were pathological complete response (pCR) rates (ypT0/is/ypN0), clinical response rates (CR+PR), and adverse events. Safety was assessed in all pts who received at least one dose of the study drug.

Results:
Between 12/2013 to 3/2016, 121 pts were randomly assigned equally to E + FEC and P + FEC. Excluding 5 pts from the primary assessment, 116 pts (58 in each group) were included in the full analysis set. The characteristics of the pts were similar in the two arms. At the end of E or P administration, the incidences of PSN were 55.4% and 92.9% in E and P arm, respectively (p<0.001). The incidences of PMN were 25.9% and 44.9% in E and P arm, respectively (p=0.049). At the end of E or P + FEC, PSN accounts for 38.9% in E arm and 85.2% in P arm (p<0.001), and PMN accounts for 20.7% in E arm and 32.8% in P arm (p=0.201). The pCR rates in E and P arm were 20.7% and 29.8% (p=0.092). The clinical response rates in E and P arm were 82.2% and 91.0% (p=0.108). No statistical significant difference was found in efficacy of PST between E and P.

Conclusion:
This randomized phase II study revealed that eribulin had favorable peripheral neuropathy profile with modest efficacy in the neoadjuvant setting, compared with paclitaxel.
2017 San Antonio Breast Cancer Symposium

Publication Number: P6-15-06

Title: SOLTI-0702 CAPRICE: Final results of a phase II study of pegylated liposomal doxorubicin plus cyclophosphamide followed by paclitaxel as neoadjuvant chemotherapy in elderly or cardiotoxicity-prone patients with high-risk breast cancer: 5-year overall survival disease free survival and late cardiac safety

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Body: Background:
Anthracycline and taxane-based chemotherapy is the standard treatment for high-risk breast cancer. However, conventional anthracyclines are not commonly used in elderly patients or those patients prone to cardiotoxicity and there is a potential risk leaving them undertreated. Pegylated liposomal doxorubicin (PLD) has comparable efficacy, but significantly less cardiotoxicity than conventional anthracyclines. We conducted a phase II trial to assess the efficacy and safety of a neoadjuvant chemotherapy (NC) based on PLD and paclitaxel (PTX) in this group of patients. The pathological complete response, breast conservative surgery (BCS) and safety data at a 35-month follow-up were published (Gil-Gil et al. Breast Cancer Res Treat 2015). Here we present the final analysis of 5-year overall survival (OS) and 5-year disease-free survival (DFS) and cardiac safety after 60 months of follow-up.

Methods:
Fifty patients with stage II (48%) and III (52%) breast cancer (seven cases were T4d) and with at least one risk factor for developing cardiotoxicity were included. NC schedule: PLD 35 mg/m² plus cyclophosphamide 600 mg/m² every 4 weeks for four cycles, followed by 80 mg/m² weekly PTX for 12. Median age was 73 years old (84% were older than 65 years). Forty-eight (96%) of tumors were triple negative (TN). Secondary objectives included 5-year DFS, 5-year OS and cardiac safety measured by a decrease in left ventricular ejection fraction (LVEF), electrocardiogram (ECG) and a cardiac questionnaire performed every 3 months during the first year, every 6 months year 2-3 and every 12 months year 4-5 of follow-up.

Results: Forty-eight patients (96%) completed the 4 cycles of PLD plus CPM, while only 26 patients (52%) could complete the 12 weeks of PTX. Forty-six patients (92%) underwent surgery. After surgery: 27 patients received radiotherapy, 2 letrozole and 1 trastuzumab. The 5-year OS was 56% (95% CI 41.2-68.4) and the 5-year DFS was 54.4% [95% CI: 38.3-67.9]. No significant decrease in LVEF was seen (mean baseline LVEF was 66.6 (52-86) and mean LVEF after 60 months was 66 (54.5-73). Four patients (8%) developed cardiotoxicity (in 2 cases G3). There were 5 non-cancer deaths (10%): 3 during treatment (all in patients > 80 years: a sudden death one month after surgery, a haemorrhagic stroke 30 days after completing chemotherapy and a non-neutropenic pneumonia); and 2 during follow-up (1 Amyotrophic Lateral Sclerosis and 1 intestinal ischemia).

Conclusions:
PLD followed by PTX as NC was feasible in a fragile population of patients who were not candidates for conventional doxorubicin. The 5 year DFS and 5 year OS in elderly patients with bulky TN tumors were similar to the reported in the literature. This regimen could be an option for the neoadjuvant treatment of cardiotoxicity-prone patients or elderly patients who present high-risk breast cancer.
Title: Pathologic complete response (pCR) in locally advanced triple negative (TN) and HER2+ (HER2+) breast cancer (BC) treated with anthracycline-free neoadjuvant therapies and associations with gene expression (GE) patterns, tandem repeats (TR), and intratumoral cellular compositions

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Body: Response to neoadjuvant therapy (NT) is a predictor of progression-free and overall survival in HER2+ and TNBC. In an attempt to decrease toxicities and increase efficacy, we designed 2 prospective anthracycline-free NT trials. Patients (Pts) with TN BC received carboplatin (carb) and nab-paclitaxel (nab); pts with HER2+ BC received pertuzumab (pert), trastuzumab (trast), and nab. Pre-NT biopsies were procured to evaluate for biological predictors of pCR.

Methods: HER2+ pts received 6 cycles (C) of pert (day 1 every 21 days [d]), and weekly trast and nab 100 mg/m². TN pts received 4 Cs of carb (AUC 6) on d 1 of a 28-d C, and weekly nab 100 mg/m². RNA extracted from formalin fixed paraffin embedded (FFPE) biopsies pre-NT was tested for Mammaprint/Blueprint and custom Agilent full genome microarrays for gene expression (GE, by Agendia Inc). The raw gMeanSignal was log2 transformed and normalized to the 75th percentile for GE analysis. Association between Blueprint/Mammaprint results and pCR was tested by Fisher exact test. The linear model from R limma package was applied. Ingenuity Pathway (PW) analysis was applied to assess functional pathways associated with pCR. Cellular distribution by Cibersort analysis was carried out to estimate the abundances of 22 different cell types in each patient sample and test whether the distribution of the cell types is different between pCR and non-responders. Tandem repeat assessment was carried out by computing a Tandem Duplicator Phenotype score, based on the number, genomic distribution and length of somatic DNA duplications identified through Whole Genome Sequencing of tumor DNA. Results: 36 of 41 HER2+ and 55 of 59 TN pts were assessed for pCR rate (too early 8, 1 ineligible). The pCR rate was 66.7% (22/36) for HER2+ pts and 51% (28/55) for the TN pts. Sufficient quality RNA and DNA were available from the first 43 of 55 pts with TNBC and 10 of 36 pts with HER2+ BC. In the 53 pts with GE assessment, pCR was inversely associated with luminal Blueprint type (P=0.04). With fold change >1.5 and P-value < 0.05; 36 genes were differentially expressed (DE) in TNBC. There were 1937 DE genes for HER2+ were narrowed down to a 67 gene signature by applying fold change > 3 and P-value <0.005, based on pCR by unsupervised clustering. CIBERSORT analysis suggested that T-cell regulatory cells (Tregs) were associated with pCR in TN BC, and 5 cell types (plasma cells, Tregs, macrophage, dendritic cells and neutrophils) presented differently between all pCR and non-pCRs with P-value <0.05. Tandem repeat analysis to assess their correlation with pCR are ongoing. Conclusions: In this analysis the non-luminal Blueprint subtype was predictive of pCR in TN and in HER2+ BC. Preliminary analysis suggested that 36-gene signature for TN and 67-gene signature for HER2+ were associated with pCR. CIBERSORT analysis revealed 5 cell types with different abundance between the pCR and non-responders, suggesting the need to target the tumor microenvironment. Results of the tandem duplicator phenotype analysis will be presented.
Title: Neoadjuvant chemotherapy vs neoadjuvant endocrine therapy in ER/PR positive HER-2 negative post-menopausal women with breast cancer, is one superior than other? A NCDB analysis

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Body: Background:
Small prospective studies show comparable response rates (RR) and breast conservation rates (BCR) among neoadjuvant endocrine therapy (NET) and neoadjuvant chemotherapy (NCT) in post-menopausal hormone receptor (HR) positive breast cancer patients. Recently we reported statistically significant differences in utilization, trends, RR, BCR and overall survival (OS) outcomes in HR positive post-menopausal women from NCDB (2004-2014) at ASCO 2017 meeting. The absolute difference in OS calculated at 5 yrs for NCT vs NET was 10.9%. However, we were not able to exclude human epidermal growth factor receptor (HER-2) positive group due to the unavailability of information. Therefore, our results might have been skewed. Thus, here we report RR, BCR and OS outcomes in post-menopausal women with HR positive, HER-2 negative breast cancer using NCDB from 2010-2014 during which HER-2 status was recorded.

Methods:
We extracted data on HR positive, HER-2 negative breast cancer patients aged ≥ 50 without metastasis from the NCDB registry (2010-2014). RR and BCR between NET and NCT was assessed using univariate and multivariate analysis. OS was calculated using Kaplan Meier analysis with hazard ratio (HR) from cox regression model. We excluded patients who did not receive adjuvant endocrine therapy after NCT and patients who received adjuvant chemotherapy after NET as this could affect OS.

Results:
Out of 25,609 breast cancer patients reported in NCDB from 2010-2014, 19,988 women met our inclusion criteria. 5759 received NET and 14,229 received NCT. On multivariate analysis NET use was higher in academic centers [Odds ratio (OR) 1.327, 95% CI 1.222-1.440], patients with age>70 (OR 6.213, 95% CI 5.615-6.875)]. NET use was lower in black race (OR 0.774, 95% CI 0.679- 0.882), tumors with higher grade (OR 0.160, 95% CI 0.141-0.181), higher T stage (OR 0.352, 95% CI 0.314-0.395), higher N stage (OR 0.177-0.246) and private insurance (OR 0.65, 95% CI 0.525- 0.806), (all p<0.0001). RR was significantly higher for patients receiving NCT (88.7%) compared to NET (77.1%), with an adjusted OR (aOR) of 2.058, however the mastectomy rate was higher in NCT (68.9%) compared to NET group (48.9%) with aOR of 1.755. OS was calculated in 15,268 women. OS rate was 98.0% vs 98.9% at 1 yr for NET vs NCT and 73.6% vs 76.9% at 5 yrs for NET and NCT respectively with adjusted hazard ratio (HR) of 1.216; 95% CI (1.072-1.380).

Conclusions:
Our analysis demonstrates higher response rate with NCT over NET even in HER-2 negative HR positive breast cancer. However, more patients underwent mastectomy in the NCT group despite high RR. Statistically significant improvement in OS was also seen with the use of NCT however the magnitude was less pronounced compared to our previous cohort which included HER-2 positive as well as negative patients. Limitations that should be considered in this registry based study are: differences in surgical technique, patient's choice, duration and choices of adjuvant therapy.
2017 San Antonio Breast Cancer Symposium

Publication Number: P6-15-09

Title: Final results of weekly (w) neoadjuvant carboplatin (Cp) added to paclitaxel (P) followed by epirubicin (E) and cyclophosphamid (C) in triple negative breast cancer (TNBC) patients (pts): A BSMO breast cancer task force phase II study

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Body: Introduction: Overall prognosis of early TNBC remains inferior to that of other breast cancer subtypes. Neoadjuvant platinum added to taxanes-anthracycline regimen has been reported to potentially improve pathologic complete response (pCR) and survival in TNBC pts.

Aim: To report the final results of the efficacy and toxicity of the addition of weekly Cp to P and dose dense (dd) EC on the pCR rate in an open-label phase II study in stage II/III TNBC pts.

Patients and methods:
In the BSMO study sixty three pts received dd P (80mg/m²/wk) concurrent with Cp (AUC=2) for 12 weeks, added to bi-weekly E (90mg/m²) and C (600mg/m²) for 4 cycles, followed by surgery and radiotherapy. The primary endpoint was pCR in the breast and axilla. Additionally actual drug dosing and toxicities were registered. A correlative assessment of germline mutations in HRD genes is ongoing. Pts were monitored for clinical response by magnetic resonance and mammography, and also for relapse free survival and time to treatment failure. The study sample size has been calculated according to the optimal Simon's two-stage design method. The target sample size was 63 patients with 80% power to detect a pCR rate of $\geq 47\%$ ($\alpha=0.05$).

Results:
Sixty three eligible pts with operable, non-inflammatory stage II/III TNBC were included. Most pts were between 40 and 59 yrs old and had clinical stage II disease. Forty percent were clinically node + and 68% were G3. Seventy three percent received breast conserving surgery. Sixty percent (38 out of 63 pts) achieved a pCR breast/axilla. Sixteen percent (10pts) missed three or more doses of wP, whereas at least 1 EC cycle was skipped in 19% (12pts). Sixty five percent had G3/4 neutropenia. Investigator reported febrile neutropenia occurred in 18 pts (28.5%) of which more than eighty percent during the EC part despite primary prophylaxis. Thrombocytopenia G3/4 was noticed in 10 pts (16%). Only four pts (6%) developed grade 3 peripheral neuropathy.

Conclusion:
The addition of weekly carboplatin to neoadjuvant paclitaxel and ddEC is feasible and a pCR rate in the breast and axilla as high as 60% compares nicely with the results achieved with the similar 3 weekly carboplatin arm of the CALBG 40603 trial. Febrile neutropenia rate was higher than the 3-weekly carboplatin arm in CALGB40603 trial, but occurred mainly during the EC part, while other toxicities are comparable. Future research could focus on this combination in the reverse sequence (first ECdd), which may lead to a better global haematological profile.
Weekly paclitaxel, pertuzumab and trastuzumab (TPH) neoadjuvant therapy for HER2 positive inflammatory breast cancer

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Body: Background: Inflammatory breast cancer (IBC) is inoperable at presentation, thus neoadjuvant systemic therapy (NAS) is the primary treatment for this aggressive disease. Due to its rarity, patients (pts) with IBC are incorporated into NAS clinical trials for locally advanced breast cancer, making it difficult to extrapolate efficacy specifically for pts with IBC. A commonly used regimen for the treatment of HER2+ IBC includes docetaxel, carboplatin, pertuzumab (P) and trastuzumab (H), yet only 6% of pts enrolled in the clinical trial for this regimen had IBC. We sought to examine the efficacy of maximizing anti-HER2 therapy combined with minimal chemotherapy using the TPH regimen specifically for pts with HER2+ IBC.

Methods: Pts with newly diagnosed HER2+ IBC received NAS with 16 weeks (wks) of paclitaxel (T) 80mg/m2/wk, H (2mg/kg/wk) and P(420mg/kg/3wk) followed by modified radical mastectomy (MRM) on a phase II prospective study. All pts had 2 research breast biopsies (rbx) for correlative assays prior to and 1 wk after the P (840mg/kg) and H (4 mg/kg) loading dose. Pts who achieved a pCR (pathologic complete response) could opt out of adjuvant doxorubicin (A) 60 mg/m2 + cyclophosphamide (C) 600mg/m2 x 4; pts with residual disease received AC. All pts received post-mastectomy radiation and maintenance P (420mg) + H (6mg/kg) every 3 wks x 12. Adjuvant endocrine therapy was given per standard of care. Primary objective was pCR rate in the breast and axillary lymph nodes. Residual Cancer Burden (RCB) was assessed. Based upon a Simon two-stage design, this regimen would be declared worthy of further study if >7/27 pCR were observed (15% vs 40%; target \( \alpha =0.039 \) power=0.90). The study was closed after 23/27 pts were enrolled due to slow accrual.

Results: 20 pts were enrolled as of 12/2016, 18 completed NAS and MRM. All but 1 had stage III disease at presentation. 1 pt was lost to follow-up; 1 developed CNS metastasis during NAS and did not undergo MRM. The mean age was 49 years, 10 pts had ER/PR negative disease. 15 pts completed 16 wks of T, 4 had 15 wks and 1 had 13 wks. During NAS, there was no grade (gd) 4 toxicity; 6 episodes of gd 3 toxicity (2 related to treatment-diarrhea); and no gd 3 cardiac events. In the intent to treat analysis, 10/20 pts achieved pCR (50%; 90% CI 30-70%) and 6 had RCB-1 (30%). 5 pts with RCB-1 response had <5 mm residual disease; 1 had lymph node involvement. Of those proceeding to MRM, pCR rate was 56% (10/18). 6/10 opted out of AC. Treatment and follow-up for clinical outcomes continue. Biologic correlates investigating genomic profiling and patterns of HER2 resistance are being performed on rbx, residual disease and cfDNA.

Conclusion: THP x 16wks is tolerable and effective NAS for HER2+ IBC, resulting in a high pCR rate with minimal toxicity. This study of NAS explored the benefit of maximizing HER2-directed therapy and minimizing chemotherapy and its associated toxicity. It has achieved its primary endpoint and will be used as the backbone NAS for HER2+ IBC, with future studies building upon this regimen. The result of this trial supports the benefit of clinical trials designed specifically for pts with IBC. Clinical trial information: NCT01796197.

2017 San Antonio Breast Cancer Symposium

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Title: Weekly paclitaxel, pertuzumab and trastuzumab (TPH) neoadjuvant therapy for HER2 positive inflammatory breast cancer
2017 San Antonio Breast Cancer Symposium

Publication Number: P6-15-12

Title: A functional approach to the molecular basis of neoadjuvant treatment response in breast cancer

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Body: BACKGROUND
Breast cancer is a diverse and heterogeneous disease. The use of neoadjuvant treatments has improved the prognosis of localized breast cancer. However, molecular basis of neoadjuvant treatment response and resistance remains unknown. Clinical data has uncovered the existence of different tumor responses to neoadjuvant chemotherapy, allowing the classification of patients in different groups. Gene expression profile description of the different patient groups provide essential information in the clinical decision making as well as to allow a deeper knowledge of this disease.

MATERIALS AND METHODS
A breast cancer tumor dataset was obtained from the Gene Expression Omnibus (GSE41998) and from a phase II trial (NCT00455533). 279 tumors from previously untreated women with primary invasive breast adenocarcinoma were included in this study. Whole genome gene expression profiling was performed using Affymetrix GeneChip gene expression microarrays.Differentially expressed genes were chosen selecting 3000 more variable probes among all patients and were used to construct four networks of gene functional interactions, one for all tumors and three for each molecular subtype independently. Functional structure was performed using probabilistic graphical models with local minimum Bayesian Information Criterion. Data analyses were carried out using MeV, BRB Array Tools, R, Cytoscape software suites and DAVID web tools.

RESULTS
Regardless of tumor molecular subtype, tumors showing a complete response to treatment showed higher "Immune response (MHCII)", "Immune response (chemotaxis)", "Immune response (B cell)" and "Immune response (Interferon)" nodes activities compared to resistant tumors (stable disease tumors). These differences are also observed when analyzing tumor molecular subgroups (Luminal A, Luminal B and Basal-like) separately. Moreover, complete response tumors, showed significantly higher levels of lymphocytic cell lineage markers (CD4, CD8 and CD20).

CONCLUSION
This type of approach allows seeing differences at biological process levels rather than at the individual gene level. Tumors that respond to neoadjuvant treatment showed higher "Immune" nodes activity than resistant tumors and these differences were also showed in analyses stratified by molecular subtype. Besides, complete response tumors presented higher values of lymphocyte cell lineage markers which might suggest a greater amount of tumor-infiltrating lymphocytes (TILs). These results can suggest that patients' immune system could play an important role in the response to neoadjuvant chemotherapy treatment.
Title: Triple negative breast cancer predisposition genes

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Body: Background: Germline cancer testing panels provide an effective method for identifying individuals at increased risk for breast cancer. However, estimates of risk for triple negative breast cancer (TNBC) (estrogen receptor-negative, progesterone receptor-negative, HER2-negative) associated with pathogenic mutations in panel genes have not been established. We sought to define the genes that contribute to TNBC.

Methods: Germline hereditary cancer multigene panel testing results were obtained for 8,753 TNBCs evaluated by a clinical testing laboratory. Associations between pathogenic mutations in individual genes and TNBC were assessed by comparing mutation frequencies in TNBCs and in the Exome Aggregation Consortium, non-Finn European, non-Cancer Genome Atlas reference controls.

Results: Inactivating mutations in 21 known cancer predisposition genes were identified in 14.6% of TNBCs. BRCA1, BRCA2, PALB2, BARD1, and RAD51D alterations were associated with high risks (odds ratio(OR)>5.0) of TNBC and variants in BRIP1, RAD51C, MSH6, and TP53 were associated with moderate risks (OR>2). In contrast, ATM, CHEK2, NBN, and RAD50 yielded no clinically relevant risks of TNBC. Pathogenic mutations in these established non-BRCA1/2 TNBC susceptibility genes were detected in 6.3% of TNBCs. Similar trends were observed among African American TNBCs. Overall, 5.5% of TNBCs with pathogenic mutations did not meet NCCN clinical testing criteria for BRCA1/2 due to a lack of significant family history and diagnosis over the age of 60.

Conclusions: The identification of genes associated with elevated risk of TNBC will improve understanding of the etiology of this aggressive form of breast cancer and inform risk management of individuals receiving panel testing. The high frequency of pathogenic variants suggests that all patients with TNBC, regardless of age of diagnosis or family history of cancer, should be considered for multigene panel testing.
2017 San Antonio Breast Cancer Symposium

Publication Number: PD1-02

Title: Cancer predisposition genes in metastatic breast cancer – Association with metastatic pattern, prognosis, patient and tumor characteristics

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Body: Background

New treatment strategies for metastatic breast cancer (mBC) are mainly driven by therapies against specific targets. BRCA mutations are one of the few established actionable targets, with PARP-Inhibitors and Platinum showing high efficacy in mBC. Hereditary cancer testing panels are now broadly used for identification of individuals with BRCA1/2 mutations who may benefit from these therapies. Many of these panels also contain other predisposition genes involved in BRCA-related DNA repair pathways, but the clinical relevance of mutations in these genes remain unclear. The aim of this study was to describe the mutation rates of BRCA1/2 and panel-based predisposition genes, and the associated clinical characteristics of individuals with these mutations, in a prospective cohort of mBC patients.

Methods

The PRAEGNANT mBC registry (NCT02338167) is a prospective registry for metastatic breast cancer patients with a focus on molecular biomarkers. Patients receiving any therapeutic regimen are eligible for this registry. Germline DNA was collected at study entry and genotyped for 37 cancer predisposition genes including BRCA1 and BRCA2. The frequency of mutations in each gene was determined, and associations between mutations and patient and tumor characteristics, metastatic pattern, and overall survival were assessed.

Results

Mutations in established high (odds ratio (OR)>5.0) and moderate risk (OR>2.0) breast cancer genes (BRCA1/2, PALB2, CHEK2, ATM, RAD51D, BARD1, and MSH6) were seen in 123 of 1462 tested patients with mBC (8.4%). BRCA1 and BRCA2 mutations were seen in 1.4% and 2.9% of patients respectively. Most frequently mutated non-BRCA panel genes were CHEK2, PALB2 and ATM with 2.8%, 0.8% and 0.6% of patients. Mutation frequency varied with regard to patients who developed brain metastases, visceral metastases or bone only metastases. BRCA1 or BRCA2 mutations were seen frequently in patients with brain (5.3%) or visceral metastases (5.2%), but were present in only 2.5% patients with bone only metastases and 1.5% of patients with lesions in other locations. Panel genes were equally distributed among all metastatic patients. PALB2 mutations (n=11) were only seen in patients with brain (1.9%) and visceral metastases (0.9%), but not in patients with bone metastases or other locations. 36.4% (N=4) of all patients with PALB2 mutations developing a brain metastasis. When adjusted for other mBC prognostic factors, a mutation (all genes) was associated with an unfavorable prognosis (HR: 1.50; 95%CI: 1.04 to 2.30, p=0.03). Mutation frequencies were similar according to therapy lines. All other associations with molecular subtypes and risk factors were similar to primary breast cancer cases.

Conclusion

Mutations in high and moderate risk breast cancer genes were frequent in metastatic breast cancer patients. The frequency was substantially higher than the 4 to 5% mutation frequency observed among unselected primary breast cancer patients, but is
consistent with recent results from studies of metastatic prostate cancer patients. Patients with brain and visceral metastases had the highest BRCA mutation rates. Results suggested that PALB2 mutations may be more frequent in patients with brain metastases.
Title: Clinically actionable pathogenic mutations that may be missed by conventional NGS-based testing: An analysis of 80,000 patients

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Body: Background: In appropriately tested patients with a personal or family history of cancer, the comprehensive assessment of inherited mutations in cancer susceptibility genes is crucial to clinical decision making. Conventional laboratory methods based on NGS (next-generation sequencing) focus on the detection of certain mutation types, notably single nucleotide variants (SNVs) and small insertions/deletions (indels), in readily accessible protein coding regions of the patients' DNA. These methods may be augmented to also detect mid-sized copy number changes (del/dup events). However, other types of clinically significant alterations can be invisible to these approaches, and for this reason their impact has been less well studied. We investigated the prevalence of such mutations in a large patient population focusing on 19 genes included in the NCCN guidelines for hereditary breast and ovarian cancer.

Methods: A diverse set of technical methods beyond conventional NGS sequencing were implemented to detect and confirm the presence of DNA alterations in over 80,000 patients. These patients were physician-referred for genetic testing and almost all had a directly relevant personal and/or family history. Pathogenicity was rigorously assessed using a method based on the ACMG 2015 guidelines. Among the technical methods used were long-read single molecule sequencing, breakpoint detection, MLPA, microarrays, and de novo methods for homopolymer associated mutations.

Results: Over 8.6% of patients with a pathogenic, potentially actionable germline mutation in one (or more) of the 19 NCCN genes harbored a mutation not easily detected by conventional sequencing or del/dup methods. In the subset of 11 NCCN genes specifically associated with breast cancer, the prevalence was slightly lower (6.8%) yet still substantial. No single class of mutation was responsible for this; rather a diversity of laboratory technical challenges were present. These included both challenging mutation types and also alterations that lie within challenging regions of these 19 genes. Data from the Precision FDA effort are consistent with these findings, showing >10-fold higher error rates for many such events when using standard NGS approaches alone.

Conclusions: Our study explored the boundaries of conventional laboratory methods and found that a significant fraction of pathogenic mutations in patients are of types that require specialized biochemical or bioinformatics methods to be used. Accurate reporting of these technically “hard” mutations is crucial to the correct diagnosis and management of cancer patients and their families, and omission of these methods can lead to high false negative rates. Based on our review of published studies, as well as a separate interlaboratory study (see associated abstract by Lincoln et al.), these methods are not yet uniformly implemented. Moreover, as some of these same genes are becoming relevant to therapeutic selection based on somatic mutation profiles, these same considerations will soon arise in tumor testing, we suspect.
Title: The contribution of rare variants, polygenic risk, and novel candidate genes to the hereditary risk of breast cancer in a large cohort of breast cancer families

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Body: Background: Identifying the missing hereditary factors underlying the familial risk of breast cancer could have a major and immediate impact on managing the breast cancer risk for these families.

Methods: We identified candidate breast cancer predisposition genes through whole exome sequencing of BRCAx families and subsequently sequenced up to 1325 genes, along with 76 common low penetrance variants associated with breast cancer, in index cases from 6,000 BRCAx families and 6,000 cancer free women (ethnically matched on principal component analysis).

Results: The role of recently described (PALB2) or suspected (MRE11A) moderately penetrant genes was confirmed. Conversely, the size of the cohort means that the absence of enrichment for loss of function (LoF mutations) provides strong evidence against other reported breast cancer genes (BRIP1, RINT1, RECQL). For further moderate risk variants (in CHEK2, ATM, BRCA2) we observed significant risk modification based on the polygenic risk score (PRS - calculated from the common variant data), with the risk restricted to the co-occurrence of the rare variant and high PRS. Novel candidate genes were identified based on LoF mutations, including NTHL1 (38 cases versus 15 controls, OR 2.5 p=0.002): a member of the base excision repair (BER) pathway. DNA sequencing of the breast carcinomas from 17 heterozygous NTHL1 mutation carriers revealed a strong bias towards a C:G>T:A (C>T) transitions, consistent with a BER defect, which confirmed the recent findings in colorectal carcinomas from bi-allelic NTHL1 mutation carriers. This data extends the cancer predisposition phenotype of NTHL1 to heterozygous carriers. In addition to NTHL1, there are a large number of candidate genes where the ratio of LoF mutations in cases versus controls indicates that they may convey an actionable level of risk; 46 genes (519 families) meet the basic criteria of multiple LoF variants and an OR >2 for cases versus controls – including previously proposed breast cancer genes MRE11A, BLM, MLH1, MYH, FANCD2 and functionally plausible candidates such as MLH3, PARP2 and ATR. Collectively the OR of breast cancer for LoF mutations in this group of genes is 3.3 (95% CI 2.7-3.9, P=3.5x10⁻⁴¹).

Conclusion: Our data shows that the effect of rare variation in established and novel breast cancer genes, along with consideration of the background polygenic risk, together explains a substantial component of the heritable risk of breast cancer in our cohort.
Title: Transgenerational epigenetic silencing of $BRCA_1$ due to a germline variant unmasks a new mechanism for familial breast and ovarian cancer

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Body: In families with multiple affected individuals with early-onset breast/ovarian cancer pathogenic variants in $BRCA_1$ or $BRCA_2$ are identified in approximately 20% of the cases. Extensive efforts have been made to identify additional highly penetrant breast cancer genes or alternative mutational mechanisms affecting $BRCA_1$ and $BRCA_2$ to explain the missing heritability. It has been proposed that part of the missing heritability may be explained by gene silencing due to promoter methylation of cancer associated genes, as described in colorectal cancer ($MLH1$ and $MSH2$). Here, for the first time, we report two independent families with multiple individuals affected by breast and ovarian cancer with transgenerational promoter methylation of $BRCA_1$. RNA analysis of $BRCA_1$ in the germline of breast/ovarian families, previously found to be $BRCA_1/2$ mutation negative by Sanger sequencing and copy number analysis, identified two families with allelic loss of expression. To investigate the mechanism of transcriptional silencing, a total of 14 affected and unaffected family members from these two families were tested for $BRCA_1$ promoter methylation by pyrosequencing in blood, buccal, and hair follicle cells. Allele specific methylation was determined by clonal bisulphite sequencing.

$BRCA_1$ promoter methylation in all three germ layers was present in 11 of 14 family members. Of the 7 women with promoter methylation five were affected with grade 3 breast/high grade serous ovarian cancer. The four males with $BRCA_1$ promoter methylation had no history of cancer. Methylation levels were ~50%, consistent with the silencing of one allele detected in RNA in these family members. Clonal bisulphite sequencing of an affected family member of each family confirmed that the alternative allele was specifically methylated. Interestingly, in both families the methylation pattern of the $BRCA_1$ promoter segregated with the same novel heterozygous variant in the 5'UTR of $BRCA_1$.

These results indicate a novel mechanism for familial breast/ovarian cancer, caused by epigenetic silencing of one allele by transgenerational hypermethylation of the $BRCA_1$ promoter, secondary to a variant in cis of $BRCA_1$. We propose that methylation analyses are indicated in all families affected by early onset breast/ovarian cancer where standard mutation screening of $BRCA_1/2$ has not identified a causative variant.
Title: Mosaic TP53 variants in women with breast cancer

Jessica L Mester¹, Kristen Postula¹, Jeffrey Bissonnette¹, Rachel T Klein¹ and Kathleen Hruska¹. ¹GeneDx, Gaithersburg, MD.

Body: Background: Germline TP53 pathogenic variants are indicative of Li-Fraumeni syndrome (LFS), a dominantly inherited hereditary cancer syndrome with high lifetime risks for female breast and other cancers. Genetic testing for LFS may reveal mosaicism, indicating a variant is present in some, but not all, of the cells tested. While these findings may represent a true constitutional event, mosaicism for TP53 variants also has been reported as a somatic event in lymphoblastoid cells from individuals with hematologic malignancy, previous chemotherapy exposure, or due to age-related clonal hematopoietic expansion. Understanding if a mosaic variant is constitutional can influence the patient's management and impact familial risk assessment. We present clinical history and follow-up testing from a series of female breast cancer patients with blood or oral rinse testing revealing a mosaic TP53 pathogenic or likely pathogenic variant (collectively, PV).

Methods: We retrospectively reviewed clinical history and genetic testing results to identify women with a personal history of breast cancer and mosaicism for one or more TP53 PV identified on multi-gene hereditary cancer testing at our clinical diagnostic laboratory. Descriptive statistics were employed.

Results: Forty-eight women with breast cancer were identified as having a mosaic TP53 PV, defined as an allelic fraction of <35%. Mean age at first breast cancer diagnosis was 49.2 years. Twenty children of 13 women with mosaic TP53 PV pursued targeted testing; none were positive for their mother's TP53 PV. Six of the 48 women (16.7%) pursued cultured fibroblast testing for the mosaic TP53 PV. In five, the PV was not found in fibroblasts. All five were diagnosed with breast cancer ≥40 years of age and three had other primary cancer diagnoses, including one with sarcoma. In one patient with early-onset and HER2-positive breast cancer, testing of fibroblasts also identified mosaicism for the TP53 PV, indicating constitutional mosaicism.

Conclusions: For individuals with a mosaic TP53 PV, identification of the variant in a second tissue is necessary to confirm constitutional mosaicism and heightened risk for other LFS-associated cancers. In this series, additional testing confirmed one of six patients with mosaic TP53 PV pursuing fibroblast testing to have constitutional mosaicism. This individual's breast cancer was HER2-positive and her age at diagnosis was younger than those whose mosaic PV was not identified in fibroblasts. Additional follow-up testing data are needed to understand whether confirmatory testing in a second tissue is indicated for any breast cancer patient with a mosaic TP53 PV, or would be most likely to reveal constitutional mosaicism in individuals whose breast cancers are HER2-positive or early-onset.
Population genetic testing for breast cancer susceptibility

Ian G Campbell¹, Simone Rowley¹, Lisa Devereux¹, Simone McInerny², Norah Grewal², Mary-Anne Young³, Amanda Lee¹, Alison Trainer² and Paul James². ¹Research Division, Peter MacCallum Cancer Ctr., Melbourne, Victoria, Australia; ²Parkville Familial Cancer Ctr, Peter MacCallum Cancer Ctr., Melbourne, Victoria, Australia and ³Garvan Institute, Sydney, New South Wales, Australia.

Background: Germline mutations in certain genes account for a large proportion of inherited risk for breast and ovarian cancer. The identification of asymptomatic mutation carriers could significantly reduce the incidence of these diseases as active risk management can dramatically reduce the risk of developing cancer. In most countries, identifying high-risk individuals is based on their family history. In general, a family is first identified because one family member develops cancer and, because of high-risk indicators is referred to a familial cancer centre (FCC). However, current data suggests that many BRCA1 or BRCA2 mutation carriers do not have a remarkable history of cancer in a close relative. Population-based genetic testing would be a far more effective strategy for identification of at-risk individuals. To test the feasibility of such a strategy we are conducting a population genetic testing trial for actionable mutations in 11 breast/ovarian cancer predisposition genes (BRCA1, BRCA2, PALB2, ATM, CDH1, PTEN, STK11, TP53, BRIP1, RAD51C, RAD51D) among 15,000 healthy women from the Australian population.

Methods: All subjects are female participants in the LifePool cohort (www.lifepool.org) who had no personal history of breast or ovarian cancer at the time of DNA collection. Participants found to carry an actionable germline mutation were notified by letter with an invitation to contact the PeterMac telephone genetic counselling service for further information and/or also invited for counselling at an FCC. Only participants with an actionable mutation were notified of their genetic testing result.

Results: Of the 5,557 women tested to date, 40 (0.72%) were carriers of mutations that are currently actionable in the Australian context (BRCA1 n=7, BRCA2 n=15, PALB2 n=15, ATM n=3). All 40 women accepted the invitation to attend a familial cancer centre for formal predictive testing. Less than 20% of the women would have met the minimum threshold for clinical genetic testing under current guidelines. A further 16 participants (0.29%) carried mutations in BRIP1, RAD51C and RAD51D but were not notified of the result as these genes are not currently actionable in Australia. No mutations were identified in CDH1, PTEN, STK11 or TP53.

Conclusions: A relatively large proportion of cancer free-women from Australia carry high-risk mutations in BRCA genes and subsequent uptake of clinical genetic testing was very high. Population-based genetic testing is well accepted and can identify a much larger proportion of the at-risk population than contemporary family history based approaches.
2017 San Antonio Breast Cancer Symposium

Publication Number: PD1-08

Title: Development and validation of a combined residual risk score to predict breast cancer risk in unaffected women negative for mutations on a multi-gene hereditary cancer panel

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Body: Background: Unaffected women with a strong family history of breast cancer (BC) are often referred for hereditary cancer testing with multi-gene panels; however, typically <10% test positive for clinically actionable mutations. Large-scale genotyping studies have identified common variants (primarily single-nucleotide polymorphisms) that individually confer modest BC risk, but together partially explain BC genetic susceptibility in many women without monogenic mutations. In addition, a number of factors relating to reproductive and medical history modify risk for BC. Here, we describe the development and validation of a combined polygenic residual risk score (cRRS) which takes into account non-genetic factors, in a large, consecutive cohort of women who tested negative for mutations in known BC susceptibility genes.

Methods: This IRB-approved study includes women of European ancestry tested with a multi-gene hereditary cancer panel who were negative for mutations in 11 genes associated with BC (BRCA1, BRCA2, TP53, PTEN, STK11, CDH1, PALB2, CHEK2, ATM, NBN, BARD1). Clinical information was collected from provider-completed test request forms. The dataset was divided into a training (July 2016– February 2017) and validation cohort (February 2017 – May 2017). 94 previously published variants (Mavaddat et al 2015; Michailidou et al 2015) were genotyped using NGS. Multivariable logistic regression models were used to evaluate the 94 variants, to develop a residual risk score (RRS) as a predictor of personal BC history in the training cohort, and to assess the performance of the RRS in the validation cohort. Independent variables included age, personal/family cancer history, and ancestry. In an additional cohort, reproductive and medical history variables will be recorded and used to calculate BC risk according to the Tyrer-Cuzick model.

Results: Accurate genotyping results were produced for 92 out of 94 variants. The training (validation) cohort included 24,259 (10,575) women, 18% (15%) of whom reported a personal history of BC. In the validation cohort RRS was strongly associated with personal history of BC (p<10^-31) with odds ratio per unit standard deviation of the RRS being 1.41 (95% CI = 1.33-1.49). The RRS outperformed a published polygenic risk score (PRS) based on 77 SNPs (Mavaddat et al 2015): in a model with both scores included, the RRS score was significantly associated with BC (p=2x10^-6) while the PRS was not (p=0.29). The RRS score also outperformed a PRS score based on the 92 variants with literature-derived odds ratios for association with BC: in a model with both scores included, the RRS score was significantly associated with BC (p=0.022) while the PRS was not (p=0.28). The RRS will be combined with the Tyrer-Cuzick model to deliver an optimized combined residual risk score (cRRS) and compared to risk predicted by Tyrer-Cuzick model alone in a separate cohort of women testing negative for BC mutations.

Conclusions: The validation and clinical implementation of a combined residual risk score for women at risk for hereditary BC may offer significant potential for the management of high-risk, unaffected women who test negative for monogenic BC mutations.
Body: Introduction: One of the preemptive strategies for Hereditary Breast and Ovarian Cancer (HBOC) is prophylactic surgery. Data for risk reducing mastectomy (RRM) clearly showed a risk reduction of more than 90% for breast cancer.

Method: We report here the statistical results of the HBOC registration up to 2016. The subjects of this study were those who underwent BRCA1/2 genetic testing during the study period, at 7 medical institutions.

Results: A total of 1527 probands underwent BRCA testing; 1125 cases (73.7%) were negative for BRCA1/2 mutation, and 297 cases (19.5%) were positive, while 105 cases (6.9%) had uncertain results. Among the 297 cases with positive results, 157 cases (10.3%) were positive for BRCA1, 139 cases (9.1%) for BRCA2, and 1 case (0.1%) was positive for both. The mean age at breast cancer diagnosis was 41.7 years in BRCA1/2 mutation positive and 45.8 years in negative cases. In comparison to the National Registration for Breast Cancer Incidence 2011 in Japan (n=72,472), breast cancer with BRCA mutations occurred at a younger age. Among 359 cases of triple negative breast cancer, 101 cases (28.3%) were BRCA1 mutation positive while 18 cases (5.0%) were BRCA2 mutation positive.

Three hundred seventy cases underwent genetic testing prior to surgery, as a deciding factor for the surgical procedure. Among BRCA mutation positive cases, 58 cases (87.9%) chose to undergo total mastectomy, and 8 cases (12.1%) chose breast conserving surgery (BCS); on the other hand, 141 cases (46.4%) of BRCA mutation negative cases chose total mastectomy and 158 cases (52.0%) chose BCS.

Four cases of new onset breast cancers were observed among the 55 cases of previvors (mean observation period: 2.5 years; incidence rate: 2.9%/Y). Among the 73 BRCA1/2 mutation positive women who underwent BCS, 3 ipsilateral breast cancer cases were observed (mean observation period: 3.5 years; incidence rate: 1.2%/Y), while 2 cases were noted among 477 cases of BRCA1/2 mutation negative cases (mean observation period: 2.2 years; incidence rate: 0.2%/Y). Of 189 BRCA1/2 mutation positive cases with unilateral breast cancer, 8 contralateral breast cancer cases were noted (mean observation period: 3.0 years; incidence rate: 1.4%/Y), while 4 cases of contralateral breast cancer were observed among 892 cases of BRCA1/2 mutation negative cases (mean observation period: 2.2 years; incidence rate: 0.2%/Y).

Fifty-one patients had undergone RRM. Six cases (11.8%) of occult breast cancer were noted in the RRM specimens, among which 2 were BRCA1 positive cases and 4 were BRCA2 positive cases. All of these six cases had undergone extensive imaging work-up prior to surgery by using mammography, ultrasound and breast MRI.

Conclusions: The incidence rate of occult cancer after risk-reducing mastectomy was reported to be about 5% in the high-risk population. Our report showed a relatively higher incidence rate of occult cancer at 11.8% among BRCA mutation positive cases, despite thorough pre-operative radiological evaluations, which included a breast MRI. These results suggest the limitations in the use of MRI for the surveillance of patients with BRCA mutations.
2017 San Antonio Breast Cancer Symposium

Publication Number: PD1-10

Title: Multi-gene panel testing results in patients with multiple breast cancer primaries

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Body: Background: Every woman diagnosed with breast cancer has a risk to develop another breast primary throughout their lifetime. This risk is significantly elevated in individuals with a BRCA1 or BRCA2 mutation. For these individuals, the risk of a second primary breast cancer is 15% within 5 years of their initial cancer and the lifetime risk could be as high as 44%. Currently, the NCCN guidelines recommend testing of the BRCA1 and BRCA2 genes for individuals with multiple breast primaries, if their first diagnosis was under the age of 50. With the advent of Next Generation Sequencing and multiple gene panels, testing for hereditary breast cancer genes outside of BRCA1 and BRCA2 has become more prevalent. Although there are currently no guidelines that dictate when multiple gene panel testing should be ordered, recent studies have shown that there may be some associations between non-BRCA breast cancer genes such as ATM, CDH1, CHEK2, PALB2, PTEN, and TP53, in individuals with multiple primary breast cancers. Our study aimed to look at our institution's cohort of individuals with multiple primary breast cancers that underwent panel testing, to determine the rates of pathogenic mutations in non-BRCA genes.

Methods: Sixty-five patients that had multiple breast cancer primaries and underwent a multi-gene breast cancer panel test were identified using MD Anderson's Clinical Cancer Genetics Database. The genetic testing results for each of these patients was reviewed and analyzed.

Results: Out of the sixty-five patients found to have multiple breast cancer primaries and had a multiple gene panel test, 23 (29%) tested positive for a pathogenic mutation in a breast cancer predisposition gene: 6 (26%) BRCA1, 3 (13%) BRCA2, 4 (17%) ATM, 3 (13%) CHEK2, 1 PALB2 (4%), 1 PTEN (4%), 1 TP53 (4%). Overall, 15% of this cohort tested positive for a mutation in a non-BRCA breast cancer predisposition gene. The average age of first breast cancer diagnosis in these positive patients was 42 (range 26-75), which was similar to the average age in patients that tested negative or were found to have a variant of uncertain significance (VUS), 42 (range26-65). The average age of the first breast cancer diagnosis in patients positive for non-BRCA breast cancer genes was 46 (range 28-75).

Conclusion: In our cohort, 29% of patients with multiple breast primaries who underwent panel testing were found to have a germline mutation in a breast cancer predisposition gene. Interestingly, 15% of our cohort were found to have a mutation in a non-BRCA breast cancer gene. There was no difference between age of onset in those that tested positive versus those that tested negative or were found to have a VUS. The high positive rate for all individuals with multiple breast cancers, regardless of age, for both BRCA1/2 and non-BRCA genes suggests that panel testing for patients with multiple breast cancer primaries should be considered. This finding should be validated in larger cohorts in order to help clarify whether all patients meeting guidelines for BRCA1 and BRCA2 genetic testing due to multiple breast cancer primaries would benefit from a multiple gene panel test.
Title: Moderate risk genes matter: Multigene testing for hereditary breast cancer

Michelle Jackson¹, Holly LaDuca¹, Jessica Profato-Partlow¹ and Carin Espenschied¹. 'Ambry Genetics, Aliso Viejo, CA.

Body: Background: Many genes have been associated with hereditary breast cancer (BC). For some genes, the associated cancer risks have been debated; however, recent studies have helped resolve some uncertainties. For example, ATM, BARD1, CHEK2, and RAD51D were associated with a moderate risk of BC (odds ratio (OR) 2- to 5-fold) in a recent study by Couch et al. Though many BC susceptibility genes have NCCN® management guidelines for at least one cancer type, some lack recommendations for BC risk management and others are missing altogether due to limited data. We aim to examine the mutation frequencies of genes on a BC focused multigene panel test (MGPT) and the possible clinical impact of these findings.

Methods: Sequential BC cases submitted to our laboratory for a BC focused MGPT between March 2012 and December 2016 were retrospectively reviewed. De-identified medical and family history data from test request forms and accompanying medical records as well as MGPT results were analyzed. Panel yield was assessed based on history of single or multiple primary diagnoses (MPD), level of BC risk, and clinical impact of test results.

Results: Of 30,161 BC cases tested, 3,174 (10.5%) were positive for a mutation, and 143 (0.5%) had more than one mutation identified. Mutations in high (>5-fold risk) and moderate risk (<5-fold risk) BC genes were detected in 3.9% and 6.6% of BC patients, respectively. Furthermore, 80.0% of the gene mutations identified (8.4% of all BC patients) occurred in genes with NCCN® management guidelines for at least one cancer type. Detection rate also varied based on personal history, with mutations detected in 10.2% of single BC cases only, 12.7% of BC cases with MPD only, and 14.0% of cases with breast and other cancers (see Table for gene specific frequencies).

<table>
<thead>
<tr>
<th>Genes</th>
<th>Breast Cancer Only N (%)</th>
<th>MPD Only N (%)</th>
<th>Breast + Other Cancers N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATM</td>
<td>246 (1.0%)</td>
<td>62 (1.5%)</td>
<td>33 (1.9%)</td>
</tr>
<tr>
<td>BARD1</td>
<td>58 (0.2%)</td>
<td>19 (0.5%)</td>
<td>3 (0.2%)</td>
</tr>
<tr>
<td>BRCA1</td>
<td>273 (1.2%)</td>
<td>65 (1.7%)</td>
<td>13 (0.8%)</td>
</tr>
<tr>
<td>BRCA2</td>
<td>336 (1.4%)</td>
<td>68 (1.8%)</td>
<td>36 (2.2%)</td>
</tr>
<tr>
<td>BRIP1</td>
<td>69 (0.3%)</td>
<td>6 (0.1%)</td>
<td>7 (0.4%)</td>
</tr>
<tr>
<td>CDH1</td>
<td>12 (0.1%)</td>
<td>5 (0.1%)</td>
<td>1 (0.1%)</td>
</tr>
<tr>
<td>CHEK2</td>
<td>412 (1.7%)</td>
<td>105 (2.6%)</td>
<td>58 (3.3%)</td>
</tr>
<tr>
<td>CHEK2 (p.I157T)</td>
<td>122 (0.5%)</td>
<td>28 (0.7%)</td>
<td>13 (0.7%)</td>
</tr>
<tr>
<td>MRE11A</td>
<td>31 (0.1%)</td>
<td>6 (0.1%)</td>
<td>2 (0.1%)</td>
</tr>
<tr>
<td>MUTYH carrier</td>
<td>312 (1.3%)</td>
<td>44 (0.1%)</td>
<td>31 (1.8%)</td>
</tr>
<tr>
<td>NBN</td>
<td>44 (0.2%)</td>
<td>5 (0.1%)</td>
<td>5 (0.3%)</td>
</tr>
<tr>
<td>NFKB</td>
<td>39 (0.2%)</td>
<td>9 (0.3%)</td>
<td>6 (0.4%)</td>
</tr>
<tr>
<td>PALB2</td>
<td>221 (0.9%)</td>
<td>43 (1.1%)</td>
<td>14 (0.8%)</td>
</tr>
<tr>
<td>PTEN</td>
<td>14 (0.1%)</td>
<td>4 (0.1%)</td>
<td>1 (0.1%)</td>
</tr>
<tr>
<td>RAD50</td>
<td>57 (0.2%)</td>
<td>8 (0.2%)</td>
<td>9 (0.5%)</td>
</tr>
<tr>
<td>RAD51C</td>
<td>32 (0.1%)</td>
<td>13 (0.3%)</td>
<td>2 (0.1%)</td>
</tr>
<tr>
<td>RAD51D</td>
<td>17 (0.1%)</td>
<td>5 (0.1%)</td>
<td>2 (0.1%)</td>
</tr>
<tr>
<td>TP53</td>
<td>47 (0.2%)</td>
<td>16 (0.4%)</td>
<td>12 (0.7%)</td>
</tr>
</tbody>
</table>
**Discussion:** Findings from this large clinical testing cohort indicate that clinicians can expect approximately 3.9% and 6.6% of BC patients to have mutations in high and moderate risk genes, respectively. Though these values may shift as gene-specific BC risks continue to be refined, it is clear that moderate risk genes play a significant role in BC susceptibility. Five genes recently added to NCCN® management guidelines (BRIP1, NBN, NF1, RAD51C, and RAD51D) accounted for 9.9% of mutations identified (1.0% of all BC cases). Including these genes in MGPT provides patients and their families clinically relevant information about cancer risks and management options. Further studies are needed on genes such as BARD1 and RAD51D that have recently been shown to predispose to BC but lack guidelines for BC risk management.
Title: Identification of copy number alterations associated with the progression of high risk premalignant breast lesions to breast carcinoma

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Body: Background: Atypical ductal hyperplasia (ADH) is common finding in the mammographic era, but has wide variation in diagnosis and treatment. ADH is a high risk factor for invasive breast ductal carcinoma (IDC), and is also considered to be a non-obligate precursor to IDC. Although a few studies have revealed some of the common genomic characteristics of ADH, a clear understanding of the molecular changes associated with breast cancer progression has been limited by inadequately powered studies and low resolution methodology (Lopez-Garcia et al. 2010, Histopathology 57: 171-192). Copy number alterations (CNAs), one of the major genetic alterations in cancer, are present in IDC and ductal carcinoma in situ (DCIS) and given a suitable detection method could be used in research and potentially in the clinical setting to predict patient prognosis.

Method: We optimised a robust, cost effective low-coverage whole genome sequencing (LCWGS) method for CNA detection, using as little as 5 ng of formalin-fixed paraffin-embedded tissue derived DNA. We applied this novel method to 21 cases of pure ADH (not associated with cancer) as well as 20 ADH with synchronous DCIS and/or IDC. Cases were reviewed independently by two pathologists, followed by micro-dissection and DNA extraction. CNA were analysed using the LCWGS method, using 5-10 ng total DNA input per sample.

Results: Highly accurate copy number profiles produced by low input LCWGS are comparable to those obtained from an alternative method, Molecular Inversion Probe arrays, while requiring 10 fold less input DNA (Kader et al., 2016, Genome Medicine 8: 121). Genetic analysis of pure ADH found that 95% had at least one copy number event and the median fraction of the genome altered was 8% (0-19%). Surprisingly, while 29% of pure ADH showed 16q loss and 1q gain (CNAs common in low-grade (LG) breast cancer), 29% of pure ADH showed 8q gain, an event more frequently observed in high grade (HG) cancer. Analysis of ADH with synchronous DCIS and/or IDC showed that 67% of ADH was clonally related with both LG (6/8) and HG carcinoma (4/7). The final analysis of 20 synchronous cases (9 LG, 11 HG) will be presented.

Conclusion: Here we report the most detailed molecular taxonomy of this high risk pre-malignant breast lesion with the largest cohort undertaken worldwide. Our observation that 67% of ADH is clonal to breast carcinoma suggests that both LG and HG carcinoma can evolve from a similar ancestor lesion. As ADH has the potential to develop both LG and HG carcinoma, a biomarker signifying breast cancer progression from pre-malignant lesions is highly desirable for the more precise treatment of individual patients diagnosed with ADH.
Title: Somatic BRCA mutation detection by circulating tumor DNA analysis in patients with metastatic breast cancer: Incidence and association with tumor genotyping results, germline BRCA mutation status, and clinical outcomes

Neelima Vidula1, Steven J Isakoff1, Andrzej Niemierko1, Giuliana Malvarosa1, Hannah Park1, Elizabeth Abraham1, Laura Spring1, Jeffrey Peppercorn1, Beverly Moy1, Leif W Ellisen1, Dejan Juric1 and Aditya Bardia1. 1Massachusetts General Hospital, Boston, MA.

Body: Introduction:
BRCA mutations may impact patient outcomes, as well as chemotherapy response in patients with breast cancer (BC). While germline BRCA mutations have been well-studied, the incidence and clinical impact of somatic BRCA mutations have not been well-described. We evaluated the presence of BRCA mutations, and the association between somatic BRCA mutations with clinical outcomes in patients with metastatic breast cancer (MBC).

Methods:
We identified patients with MBC who underwent ctDNA testing by Guardant360 at our institution before the start of a new therapy. From this subset of patients, we subsequently identified those patients with circulating tumor DNA (ctDNA) BRCA 1 or 2 mutations. We conducted a retrospective review of medical and pathology records to identify tumor subtype, germline BRCA testing results, and tissue genotyping results based on institutional Snapshot-NGS genotyping assay. In addition, we conducted a multivariate analysis to evaluate the hazard ratio (HR) for the association between ctDNA BRCA mutation and progression free survival (PFS) adjusting for age, number of prior therapies, and type of therapy.

Results
Among patients with MBC (N = 178), 27 (15.2%) had BRCA alterations detected by ctDNA analysis. Among patients with ctDNA BRCA alterations, the median age at metastatic diagnosis was 53; 16/24 (66.6%) had hormone receptor (HR)+/HER2- BC, 5/24 (20.8%) had triple negative (TN) BC, 2/24 (8.3%) had HR-/HER2+ BC, and 1/24 (4.2%) had HR+/HER2+ BC. Of patients with ctDNA BRCA mutations, only a minority (16.7%) had BRCA alterations detected by genotyping of archival tumor, and only 1 (3.7%) had a germline BRCA mutation (BRCA 1). In multivariate analysis, patients with BRCA mutant tumors, had similar median PFS as compared to non-BRCA mutant breast cancer (HR: 1.17; p = 0.58). Overall survival analysis and impact of BRCA mutations on response to therapy, particularly DNA damaging agents, will be presented at the meeting.

Conclusions:
BRCA mutations by ctDNA are detectable in a significant proportion of MBC patients. Most BRCA mutations detected by ctDNA were not identified by genotyping of archival tissue, and were not associated with germline BRCA mutations, suggesting that somatic BRCA mutations may be detected by sensitive blood-based genotyping assays in patients who are not known BRCA carriers. The therapeutic impact of DNA damaging agents and PARP inhibitors in MBC patients with somatic BRCA alterations is not known and warrants additional research.
**Title:** Induction of epigenetic BRCAness in BRCA1 wild-type triple negative breast cancer: BET inhibition as a therapeutic strategy

Lorenzo Gerratana\(^1,2\), Fabio Puglisi\(^1,3\), Giuseppe Damante\(^1,4\) and Catia Mio\(^1\). \(^1\)The University of Udine, Udine, Italy; \(^2\)University Hospital of Udine, Udine, Italy; \(^3\)CRO Aviano National Cancer Institute, Aviano, Italy and \(^4\)Institute of Genetics, University Hospital of Udine, Udine, Italy.

**Body:**

**Background:** Pharmacological induced BRCAness and synthetic lethality could represent a potential therapeutic strategy in triple negative breast cancer (TNBC). Bromodomain and Extra-Terminal proteins (BET) such as BRD2 and BRD4, are involved in controlling RNA polymerase II pause/release transition mediating transcriptional activation and gene bookmarking. BRD4 is found to be associated to enhancer and promoter regions to foster gene expression. Furthermore, BRD4 overexpression is described to be associated with worse outcome and increased metastasizing potential. Aim of this study was to explore novel therapeutic strategies through pharmacological induced epigenetic BRCAness in BRCA1 wild-type TNBC cells by means of BET inhibition.

**Methods:** The experimental models were based on the BT-549, MDA-MB-157 and MDA-MB-231 TNBC BRCA1 wild type cell lines. Two different pan-BET inhibitors were explored (i.e. JQ1 and I-BET762). BRCA1 and RAD51 levels were evaluated by both qPCR and western blotting as a marker of BRCAness phenotype onset. Chromatin immunoprecipitation (ChIP) and RNA interference (RNAi)-mediated BRD4 silencing were performed on TNBC cells. Synthetic lethality induced by JQ1 (0.5 µM) and the effects of co-treatment with the PARP inhibitor PJ34 (5-15 µM) or cisplatin (2-10 µM) were evaluated by methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay.

**Results:** A dose-dependent reduction in BRCA1 and RAD51 protein levels was observed after treatment with both JQ1 and I-BET762 in all the cell lines tested. To verify the direct relationship between BRD4 and BRCA1/RAD51 expression, a dual approach was performed. First, BRCA1 and RAD51 protein levels were evaluated after RNAi-mediated BRD4 knockdown, verifying that the BRCAness phenotype (exemplified by BRCA1 and RAD51 protein downregulation) was due to the absence of BRD4 protein. Furthermore, a ChIP assay was performed to confirm the direct regulation of BRCA1 and RAD51 promoters by BRD4. To test the gain in sensitivity of BRCAness-displaying TNBC cells towards platinum salts, the effects of cisplatin alone or in combination with BET inhibitors were tested. JQ1 and cisplatin combinations induced a strong reduction in TNBC cell viability in all the three cell lines tested. Moreover, the pharmacological combination between JQ1 and PJ34 displayed a significant cell viability decrease in TNBC wild type cells, corroborating the synthetic lethality mechanism.

**Conclusions:** A decrease in BRCA1 and RAD51 levels was observed after BET inhibition by means of the JQ1 drug, exploiting synergism with PARP inhibition and use of platinum salts. Moreover, the present results demonstrate the direct interaction between BRD4, BRCA1 and RAD51, supporting the therapeutic rationale behind this novel strategy.
2017 San Antonio Breast Cancer Symposium

Publication Number: PD1-15

Title: The landscape of somatic genetic alterations in breast cancers from ATM germline mutation carriers

Britta Weigelt¹, Rui Bi¹, Rahul Kumar², Paul A James², Heather Thorne³, Fergus J Couch⁴, Diana M Eccles⁵, Fiona Blows⁶, Felipe C Geyer¹, Anqi Li¹, Pier Selenica¹, Raymond S Lim¹, Pedro Blecua⁶, Ronglai Shen⁴, Hannah Wen¹, Mark E Robson⁸, Jorge S Reis-Filho¹ and Georgia Chenevix-Trench⁹.

¹Memorial Sloan Kettering Cancer Center, New York, NY; ²Peter MacCallum Cancer Centre, Melbourne, Australia; ³Mayo Clinic, Rochester, MN; ⁴University of Southampton, Southampton, United Kingdom; ⁵University of Cambridge, Cambridge, United Kingdom; ⁶Memorial Sloan Kettering Cancer Center, New York, NY; ⁷Memorial Sloan Kettering Cancer Center, New York, NY and ⁹QIMR Berghofer Medical Research Institute, Brisbane, Australia.

Body: Introduction: Pathogenic and/or founder germline variants in the ataxia-telangiectasia mutated (ATM) gene confer an increased breast cancer (BC) risk. The protein kinase ATM plays a central role in DNA double-strand break-repair and in the activation of downstream targets such as p53 and BRCA1. We sought to define the repertoire of somatic genetic alterations of BCs from patients with pathogenic germline ATM mutations and whether somatic loss of heterozygosity (LOH) of ATM would be present in these cancers.

Methods: 21 BCs from ATM germline mutation carriers were microdissected. Tumor and normal DNA samples were subjected to whole-exome sequencing (WES, n=12) or massively parallel sequencing targeting all coding regions and selected intronic and regulatory regions of 410 key cancer genes (n=9). Somatic mutations, copy number alterations, cancer cell fractions, large-scale state transitions (LSTs) and mutational signatures were defined using state-of-the-art bioinformatics algorithms. ABSOLUTE and FACETS were employed to assess LOH of the wild-type allele of ATM.

Results: Of the patients included in this study, 71%, 24% and 5% of cases harbored ATM missense (all but one p.V2424G), frame-shift and nonsense germline mutations, respectively. All tumors were ER-positive and four (19%) were HER2-positive. The median age of the patients was 46 years (32–79 years). Our analyses revealed biallelic inactivation of ATM through LOH of the wild-type allele in 16 of 21 cases (76%), and second somatic ATM mutations were not found. The median number of non-synonymous somatic mutations was 38 (range 15-113) and 2 (range 0-8) in tumors subjected to WES and targeted sequencing, respectively. The repertoire of somatic genetic alterations of ATM-associated BCs was found to be heterogeneous, including clonal PIK3CA mutations (24%), GATA3 mutations (19%), FANCI amplifications (19%) and CCND1 amplifications (14%). Importantly, however, no somatic mutations affecting TP53 were found. Analysis of the WES data revealed that 5 (42%) ATM-associated BCs displayed high LST scores, all of which harbored bi-allelic ATM inactivation. In contrast to BRCA1- and BRCA2-associated BCs, which frequently display the mutational signature 3 associated with defective homologous recombination DNA repair, the ATM-associated BCs studied displayed the ageing mutational signature (i.e. signature 1). Comparison of the mutational profiles of the ATM–associated BCs subjected to WES (n=12) with those of BRCA1- (n=11) and BRCA2-associated (n=10) BCs from The Cancer Genome Atlas revealed that TP53 was more frequently mutated in BCs from BRCA1 germline mutation carriers (0% vs 72%, P<0.001), while no differences with BRCA2-associated BCs were found.

Conclusion: ATM-associated BCs frequently display bi-allelic ATM inactivation through LOH of the wild-type allele and a subset of these cases displayed high levels of LSTs. These findings suggest that at least in a subset of ATM-associated BCs, biallelic inactivation of ATM rather than a dominant negative effect of the germline mutation may be the mechanism of inactivation of this tumor suppressor gene. The repertoire of somatic genetic alterations of ATM-associated BCs is heterogeneous, with a noticeable lack of TP53 somatic mutations.
Title: Ultrasound together with clinical indexes cannot predict sentinel lymph node metastasis for ultrasound-axillary lymph node-negative breast cancer patients

Xue Chen¹, Yingjian He¹, Ling Huo¹, Jinfeng Li¹, Yuntao Xie¹, Tianfeng Wang¹, Zhaoqing Fan¹ and Tao Ouyang¹. ¹Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Breast Cancer Prevention & Treatment Center, Peking University Cancer Hospital & Institute, Beijing, China.

Body: Background: Sentinel lymph node (SLN) pathology result is crucial to predict axillary lymph nodes (ALN) metastasis as well as to determine systemic treatment strategy. Ultrasound has been paid great attention to the evaluation of ALN metastasis. Whether the combination of known clinic-pathological indexes to ultrasound could predict SLN metastasis for ultrasound-ALN-negative breast cancer, and ultimately achieve the goal of avoiding the invasive method of sentinel lymph node biopsy (SLNB), is the current focus.

Objective: To discuss the possibility of predicting SLN metastasis using axillary ultrasonography in combination with patients' clinic-pathologic factors by retrospectively analyzing our institution's large cohort of ultrasound-ALN-negative breast cancer patients' data.

Method: This study collected consecutive data from the prospective database of Breast Center of Beijing Cancer Hospital from Oct. 2010 to Apr. 2016. Inclusion criteria: Pathologically diagnosed as primary breast cancer by core needle biopsy (CNB); negative ALN by ultrasound (no ALN detected, or the cortex thickness was even and <3mm); no treatment prior to SLNB. The SLN pathological outcomes were correlated with known clinic-pathologic parameters. Univariate analysis was performed by Chi-Square test, with \( p < 0.05 \) considered as statistically significant difference. Logistic regression analysis was used for the multivariate analysis, the area under curve >0.75 stands for acceptable predicting accuracy.

Results: Non-selective consecutive data with a total of 4,936 primary breast cancer cases treated from Oct. 2010 to Apr. 2016 was extracted from the prospective database. Exclusion criteria: Pathologically diagnosed by surgical resection (n=492); carcinoma in situ (n=145); abnormal ALN by ultrasound underwent fine needle aspiration (FNA) or CNB (n= 750); systemic treatment prior to SLNB (n=349); no SLN detected after injection (n=81); male (n=4). A total of 3,115 cases met the inclusion criteria. Among which 2,317 (74.3%) cases were negative SLN pathology and 798 (25.7%) cases were positive SLN pathology. The main findings of this study were that the univariate analysis such as, patients' age, menstruation, tumor size, ER/PR, HER-2 were influential factors, \( p < 0.05 \). Multivariate analysis showed that the area under the ROC curve was 0.658 (95% CI 0.637-0.679), indicating that the combination of all the clinic-pathologic factors with ultrasound could not stand for acceptable predicting accuracy.

Conclusion: Ultrasound together with clinical indexes cannot predict SLN metastasis for ultrasound-ALN-negative breast cancer patients.

The result of univariates related to SLN

<table>
<thead>
<tr>
<th>Items</th>
<th>SLN-Negative%(n)</th>
<th>SLN-Positive%(n)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age≤40</td>
<td>70.5(324)</td>
<td>29.5(136)</td>
<td>0.036</td>
</tr>
<tr>
<td>&gt;40</td>
<td>75.1(1,993)</td>
<td>24.9(662)</td>
<td></td>
</tr>
<tr>
<td>Premenopausal</td>
<td>71.8(1,215)</td>
<td>28.2(477)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>77.4(1,102)</td>
<td>22.6(321)</td>
<td></td>
</tr>
<tr>
<td>T size(cm)≤2</td>
<td>78.4(1,242)</td>
<td>21.6(342)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T size(cm)&gt;2</td>
<td>70.2(1,075)</td>
<td>29.8(456)</td>
<td></td>
</tr>
<tr>
<td>IDC I</td>
<td>83.0(455)</td>
<td>17.0(93)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IDC II+III</td>
<td>71.1(1,603)</td>
<td>28.8(649)</td>
<td></td>
</tr>
<tr>
<td>Other pathology types</td>
<td>82.2(259)</td>
<td>17.8(56)</td>
<td></td>
</tr>
<tr>
<td>ER≤10%</td>
<td>85.8(652)</td>
<td>14.2(108)</td>
<td>&lt;0.001</td>
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<td></td>
</tr>
<tr>
<td><strong>ER&gt;10%</strong></td>
<td>70.7(1,665)</td>
<td>29.3(690)</td>
<td></td>
</tr>
<tr>
<td><strong>PR≤10%</strong></td>
<td>80.3(851)</td>
<td>19.7(208)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>PR&gt;10%</strong></td>
<td>71.3(1,466)</td>
<td>28.7(590)</td>
<td></td>
</tr>
<tr>
<td><strong>HER-2 0,1+,2+&amp;FISH-</strong></td>
<td>73.1(1,729)</td>
<td>26.9(635)</td>
<td>0.008</td>
</tr>
<tr>
<td><strong>HER-2 3+,2+&amp;FISH+</strong></td>
<td>78.3(588)</td>
<td>21.7(163)</td>
<td></td>
</tr>
</tbody>
</table>
**Title:** Indocyanine green fluorescence-guided video-assisted sentinel node biopsy: A prospective comparative study and cost-analysis

Luca Sorrentino¹, Serena Mazzucchelli², Marta Truffi², Gaia Pietropaolo¹, Alessandra Sartani¹, Diego Foschi¹² and Fabio Corsi²³.

¹University of Milan, "Luigi Sacco" Hospital, Milan, Italy; ²University of Milan, "Luigi Sacco" Hospital, Milan, Italy and ³IRCCS Maugeri Foundation Hospital, Pavia, Italy.

**Body:** Background: Currently the standard techniques for sentinel node (SLN) detection in breast cancer are radioisotope (RI) and blue dye, but both methods present some drawbacks. Indocyanine green (ICG) fluorescence has been recently proposed as an alternative technique. However, the equipment to detect ICG fluorescence is not widely accessible, limiting the potential and the diffusion of this encouraging technique. The aim of this study was to assess the feasibility, accuracy and healthcare costs of a novel approach for SLN biopsy by a video-assisted ICG-guided technique.

**Methods:** A prospective study was performed enrolling 335 breast cancer patients: SLN was detected with RI in 194 cases, with ICG in 70 cases, and with ICG plus RI in 71 cases. ICG fluorescence was detected using a laparoscope with a near-infrared filter, and a video-assisted SLN biopsy was performed by approaching the camera in the axillary cavity. Detection rates were compared between ICG and RI. Healthcare costs were analyzed considering surgery and hospitalization times, stratified by type of surgery.

**Results:** In ICG + RI group, ICG detected 90.9% of metastatic SLNs, while RI and ICG + RI detected 100% of them. Detection rate was 100% with ICG + RI, 95.1% with RI and 92.7% with ICG. More SLNs per patient were identified with ICG and ICG + RI compared to RI (<0.0001). Healthcare costs were equivalent among the 3 groups.

<table>
<thead>
<tr>
<th></th>
<th>ICG (n = 70)</th>
<th>RI (n = 194)</th>
<th>ICG + RI (n = 71)</th>
<th>p value (ICG vs. RI)</th>
<th>p value (RI vs ICG + RI)</th>
<th>p value (ICG vs ICG + RI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of identified sentinel nodes</td>
<td>80</td>
<td>195</td>
<td>82</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients with 1 SLN</td>
<td>61 (87.1%)</td>
<td>193 (99.5%)</td>
<td>61 (85.9%)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>1</td>
</tr>
<tr>
<td>Patients with &gt;1 SLN</td>
<td>9 (12.9%)</td>
<td>1 (0.5%)</td>
<td>10 (14.1%)</td>
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</tr>
<tr>
<td>Missing of metastatic SLN</td>
<td>Yes</td>
<td>1 (1.4%)</td>
<td>0 (0.0%)</td>
<td>0.27</td>
<td>1</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>69 (98.6%)</td>
<td>194 (100.0%)</td>
<td></td>
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<tr>
<td>SLN status</td>
<td></td>
<td></td>
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<tr>
<td>Negative/ITC</td>
<td>63 (77.8%)</td>
<td>149 (76.4%)</td>
<td>71 (86.6%)</td>
<td>0.34</td>
<td>0.17</td>
<td>0.23</td>
</tr>
<tr>
<td>Micrometastasis</td>
<td>5 (6.2%)</td>
<td>22 (11.3%)</td>
<td>5 (6.1%)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Macrometastasis</td>
<td>13 (16.0%)</td>
<td>24 (12.3%)</td>
<td>6 (7.3%)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Mastectomy + SLN biopsy + Axillary dissection</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Operative times (min)</td>
<td>113.3 (±30.1)</td>
<td>139 (±32.3)</td>
<td>123.3 (±25.2)</td>
<td>0.25</td>
<td>0.46</td>
<td>0.68</td>
</tr>
<tr>
<td>Hospitalization (days)</td>
<td>4.7 (±1.2)</td>
<td>6.7 (±1.7)</td>
<td>5.7 (±1.5)</td>
<td>0.09</td>
<td>0.38</td>
<td>0.42</td>
</tr>
<tr>
<td>Total cost per patient</td>
<td>4,145.2€ (±787.9€)</td>
<td>5,723€ (±1,099.9€)</td>
<td>5,005.2€ (±966.3€)</td>
<td>0.04</td>
<td>0.33</td>
<td>0.29</td>
</tr>
<tr>
<td>Mastectomy + SLN biopsy</td>
<td></td>
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<td></td>
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<tr>
<td>Operative times (min)</td>
<td>119.4 (±33.9)</td>
<td>89.3 (±32.1)</td>
<td>103.8 (±18.5)</td>
<td>0.05</td>
<td>0.25</td>
<td>0.27</td>
</tr>
<tr>
<td>Hospitalization (days)</td>
<td>4.6 (±1.2)</td>
<td>4.6 (±0.7)</td>
<td>3.9 (±1.4)</td>
<td>1</td>
<td>0.12</td>
<td>0.3</td>
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<tr>
<td>Total cost per patient</td>
<td>4,123.3€ (±794.9€)</td>
<td>4,053.6€ (±494.6€)</td>
<td>3,731.4€ (±896.5€)</td>
<td>0.79</td>
<td>0.28</td>
<td>0.37</td>
</tr>
<tr>
<td>Procedure</td>
<td>Operative times (min)</td>
<td>Hospitalization (days)</td>
<td>Total cost per patient</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Lumpectomy + SLN biopsy + Axillary dissection</td>
<td>96.9 (±28.9)</td>
<td>4 (±1.5)</td>
<td>3,589.9€ (±971.1€)</td>
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<tr>
<td></td>
<td>111.5 (±31.8)</td>
<td>4.7 (±1.6)</td>
<td>4,268€ (±1,037.2€)</td>
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<tr>
<td></td>
<td>92 (±10.4)</td>
<td>4 (±2)</td>
<td>3,714.6€ (±1,269.9€)</td>
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<tr>
<td></td>
<td>0.26</td>
<td>0.28</td>
<td>0.11</td>
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<td></td>
<td>0.19</td>
<td>0.39</td>
<td>0.30</td>
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<tr>
<td></td>
<td>0.73</td>
<td>1</td>
<td>0.85</td>
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<tr>
<td>Lumpectomy + SLN biopsy</td>
<td>70.9 (±32.3)</td>
<td>2.2 (±0.9)</td>
<td>2,271.9€ (±611.4€)</td>
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<tr>
<td></td>
<td>67.4 (±17.9)</td>
<td>2.2 (±0.6)</td>
<td>2,383.1€ (±399.4€)</td>
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<tr>
<td></td>
<td>66.8 (±20.4)</td>
<td>2.1 (±0.4)</td>
<td>2,338.6€ (±289.1€)</td>
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<tr>
<td></td>
<td>0.34</td>
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<td>0.43</td>
<td>0.46</td>
<td>0.47</td>
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</tbody>
</table>

**Conclusions:** Video-assisted ICG fluorescence-guided SLN biopsy is a feasible technique with comparable efficacy compared to RI. Combining ICG and RI resulted in a significantly higher detection rate and identification of more SLNs per patients, providing a more accurate staging of the axilla.
Sentinel lymph node mapping with dual tracer combination: Fluorescent fluorescein with methylene blue compared to radioactive sulphur colloid with methylene blue: A randomised comparison

Anurag Srivasatava1, Jayesh Suresh1, Piyush Ranjan1, Atish Kumar1, Kamal Kataria1, Anita Dhar1 and Seenu Vathulru1. 1All India Institute of Medical Sciences, New Delhi, Delhi, India.

Objective-Fluorescein and methylene blue (FWMB) combined tracer is non-inferior to Tc99- Sulphur colloid and methylene blue (TWMB) in identification of sentinel lymph nodes in women with operable breast cancer.

Rationale-Sentinel node biopsy (SNB) is the standard procedure for axillary nodal evaluation. Most authors recommend dual tracers, a radioactive dye tagged with large particulate matter like albumin or sulphur colloid and blue dye like lymphazurin, methylene blue or patent blue for identification of sentinel lymph nodes. In Asian countries like India, the availability of radioactive pharmacetical and gamma probe facility are sparse. Hence, we have compared a combination of fluorescein and methylene blue with technetium and methylene blue in a two arm randomized controlled trial with non-inferiority hypothesis and 1:1 allocation ratio on 81 patients with operable breast cancer. The non inferiority margin was set at 5 %. The study was conducted in All India Institute of Medical Sciences, New Delhi from June 2015 to November 2016. The study included ladies with operable breast cancer of all histological types after getting informed written consent.

Novelty- This is the first randomized trial comparing fluorescein with technetium sulphur colloid in sentinel lymph node biopsy in breast cancer.

In the fluorescein and methylene blue arm, one ml of 20% fluorescein diluted with 4 ml of normal saline was injected in the periareolar region, half the dye injected intradermally and half in the sub areolar plane. One ml of 1% Methylene blue dye diluted in 4 ml normal saline was also injected in similar manner. It was followed by gentle massage for 5 minutes. An incision was made on lateral axillary skin crease and fluorescent or blue lymphatics dissected to the level of sentinel lymph nodes. Fluorescent lymphatics were identified with a blue light lamp (480 nm). Sentinel lymph node(s) were excised and sent for histopathological examination. Radioactive node was picked up by gamma probe.

Results- The base line patient and tumour characteristics of the two groups showed no significant difference. Since it was a non inferiority trial, we performed per protocol analysis revealing the identification of sentinel nodes = 84.6% in both arms (effect size-zero and 90% confidence interval -13.4 to +13.4.). Results of subgroup analysis before and after chemotherapy and SNB done under local and general anaesthesia has been given in tables 1 and 2.

<table>
<thead>
<tr>
<th>TWMB</th>
<th>FWMB</th>
<th>Effect size (ninety percent confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>33/39(84.6)</td>
<td>33/39(84.6)</td>
</tr>
<tr>
<td>Prechemo patients</td>
<td>26/29(89.7)</td>
<td>26/30(86.7)</td>
</tr>
<tr>
<td>Postchemo patients</td>
<td>7/10(70)</td>
<td>7/9(77.8)</td>
</tr>
<tr>
<td>Local Anaesthesia</td>
<td>23/25(92.0)</td>
<td>22/23(95.7)</td>
</tr>
<tr>
<td>General Anaesthesia</td>
<td>10/14(71.4)</td>
<td>11/16(68.8)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TWMB</th>
<th>FWMB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>33/39(84.6)</td>
</tr>
<tr>
<td>Prechemo patients</td>
<td>26/29(89.7)</td>
</tr>
<tr>
<td></td>
<td>Postchemo patients</td>
</tr>
<tr>
<td>--------------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td></td>
<td>7/10(70)</td>
</tr>
<tr>
<td></td>
<td>7/9(77.8)</td>
</tr>
</tbody>
</table>

Conclusion- Fluorescein + methylene blue offer an attractive cheap method of SNB.
Enhanced pre-operative axillary staging using intradermal microbubbles and contrast-enhanced ultrasound (CEUS) to identify and biopsy sentinel lymph nodes (SLN) in breast cancer is a reproducible technique and may characterise a group of patients who can completely avoid axillary surgery.

Karina L Cox¹, Nisha Sharma², Sian Taylor-Phillips³, Jennifer Weeks¹, Pippa Mills¹, Adrian Lim⁵, Isobel Haigh³, Ali Sever⁴, Matthew Wallis⁶, Tania DeSilva⁷ and Mohamed Hashem¹. ¹Maidstone and Tunbridge Wells NHS Trust, Maidstone, Kent, United Kingdom; ²WMS - Population Evidence and Technologies, University of Warwick, Coventry, United Kingdom; ³Leeds Breast Unit, Leeds, Yorkshire, United Kingdom; ⁴Breast Care Unit, Kings College Hospital, London, United Kingdom; ⁵Charing Cross Hospital, London, United Kingdom; ⁶Addenbrooke's Treatment Centre, Cambridge, United Kingdom and ⁷School of Surgery, KSS, London, United Kingdom.

**Body: Purpose**

In patients with breast cancer, avoiding overtreatment of the axilla without compromising oncological outcomes is an important clinical goal. Previous work has suggested that patients with a normal grey-scale ultrasound and benign radiological core biopsy of SLN identified with CEUS are unlikely to have high volume axillary metastases. We therefore assessed the reproducibility of this biopsy technique in multiple centres and in 2 centres, measured the volume of axillary metastases at the end of primary surgical treatment in patients with a false negative SLN core biopsy.

**Materials and Methods**

Between 2010 and 2016 data were collected on patients with early breast cancer; 1361 from Maidstone Breast Clinic (1) (prospective, sequential), 376 from Tunbridge Wells Breast Clinic (2) (retrospective, sequential), 122 from Leeds Breast Clinic (3) (retrospective, selected) and 48 from Imperial College Healthcare (4) (prospective, selected). Patients at Centres 1 and 2 had a normal grey-scale axillary ultrasound. Patients had a CEUS SLN core biopsy procedure prior to axillary surgery (sentinel lymph node excision (SLNE)/axillary lymph node dissection (ALND)).

**Results**

SLN were successfully core biopsied (nodal tissue retrieved) in 80% (Centre 1), 79.5% (Centre 2), 77.5% (Centre 3) and 88% (Centre 4). Patients with invasive breast cancer and a successful SLN core biopsy went on to have primary surgical treatment, 816 (Centre 1), 215 (Centre 2), 80 (Centre 3) and 38 (Centre 4). As a test to identify all SLN metastases, the sensitivities were 47.5% (95% CI 39.9-55.1), 52.5% (95% CI 39.1-65.7), 46.4% (95% CI 27.5-66.1) and 45.5% (95% CI 16.7-76.6) respectively. Specificities; 99.7% (95% CI 98.9-100), 98.1 (95% CI 94.5-99.6), 100% (95% CI 93.2-100%) and 96.3% (95% CI 81-99.9) respectively. Negative predictive values; 87.3% (95% CI 84.6-89.6), 84.5% (95% CI 78.4-89.5), 86.9% (95% CI 82.4-90.3) and 86.2% (95% CI 78.4-91.5) respectively. At Centres 1 and 2, 13/637 (2%) and 6/183 (3%) respectively of patients with a benign microbubble/ CEUS SLN core biopsy had 2 or more LN macrometastases found at SLNE/ ALND.

**Conclusion**

The identification and biopsy of SLN using CEUS is a reproducible technique. Despite the low sensitivity, the negative predictive value is high and in a large cohort of patients from centres 1 and 2, only a small proportion of patients had 2 or more Lymph node macro metastases that were both occult on grey-scale ultrasound and missed by SLN core biopsy. In the era of axillary conservation, these results indicate that some patients may be suitable for complete radiological staging of the axilla and thus safely avoid axillary surgery.
Evaluation of sentinel lymph nodes with high-frequency ultrasound: Correlations to histopathology

Sarah Khelfa¹, Rachel E Factor², Dolly A Sanjinez¹, Andrea F Zambrana¹ and Timothy E Doyle¹. ¹Utah Valley University, Orem, UT and ²University of Utah, Salt Lake City, UT.

Purpose: The sentinel lymph node (SLN) is biopsied during breast conservation surgery (BCS) since it is the first site of metastasis for breast cancer. If malignant, axillary lymph node dissection (ALND) may be performed to remove additional nodes in and around the affected breast, limiting the spread of the malignancy. Often, ALND results in hardship for the patient in the form of further surgeries, having nodes removed that may not be malignant, and in debilitating side effects. Developing a method to analyze SLNs intraoperatively would eliminate additional surgeries and the patient's associated suffering. This research aims to develop the use of high-frequency (HF) ultrasound (20-80 MHz) as a real-time analysis method to determine SLN status during BCS.

Background: HF ultrasonic data from BCS tissue specimens were gathered from 73 patients at the Huntsman Cancer Institute, Salt Lake City, UT, immediately following surgery. In addition to 349 margin specimens, data were collected from 78 SLNs, with initial results displaying high statistical measures. A limitation of the SLN analyses, however, was that pathology results were provided only on a per sample basis, whereas the ultrasonic method often tested multiple positions on a node (max = 4, avg = 1.26). Because of the mismatch between the ultrasonic measurements (per position basis) and pathology results (per specimen basis), an ambiguity existed in how to best analyze the data. The aim of this study was to examine the scope of this ambiguity by determining the differences in statistical measures obtained by analyzing the SLN data on a per position basis versus a per specimen basis given the current data available.

Method: HF ultrasound parameters extracted from the data were peak density (the number of peaks in the ultrasonic spectra) and attenuation. Both parameters correlate to tissue malignancy, and were used in a multivariate analysis to provide the final results. The statistical measures for the ultrasonic test results were calculated as follows:

1. per position basis: the pathology of each position was determined by the pathology results for the entire specimen;
2. per specimen basis: only one measurement position on each node was selected, based on the highest peak density value, to correlate to the specimen pathology.

Results: The analyses revealed that the HF ultrasonic data yielded an accuracy, sensitivity, and specificity of 79.6%, 76.9%, and 80.0%, respectively, for the per position basis, and 84.6%, 87.5%, and 84.3%, respectively, for the per specimen basis. The results indicate that HF ultrasound provides intraoperative detection capabilities competitive with many SLN evaluation methods currently in use, including imprint cytology, frozen-section analysis, and qRT-PCR. Detailed analyses of the SLN pathology slides from the 73-patient study are currently being conducted to improve the correlations between the ultrasound results and histopathology. Image analysis methods are being used to quantify the extent of the malignant tissue in each SLN. This will provide pathology results on a per position basis, and thus more accurate, one-to-one correlations. These correlations would significantly further the development of HF ultrasound for real-time SLN evaluation.
Title: Preoperative diagnosis of sentinel lymph node metastasis using computed tomography lymphography for early breast cancer patients

Hajime Abe¹, Atsuko Teramoto¹, Masahito Tanaka¹, Keiichi Yamasaki¹ and Kohri Yoneda¹. ¹Bell Land General Hospital, Sakai, Osaka, Japan.

Body: Background: Sentinel lymph nodes (SLN) biopsy has been established as a standard of care in the treatment of early breast cancer. This technique represents a minimally invasive, highly accurate method of axillary staging and is an alternative to conventional axillary lymph node dissection. However, an indication of SLN navigation to metastatic disease may lead to misdiagnosis for staging. Recently, SLN identification using computed tomography lymphography (CTLG) has been reported in Japan. This study investigated the value of CTLG for preoperative prediction of SLN status in early breast cancer patients.

Patients and method: Between January 2013 and August 2016, 350 breast cancer patients without clinical evidence of lymph node metastasis were treated. On the day before the operation, CTLG was performed using 64-row multidetector helical CT scanner. Patients were placed in a supine position with their arms positioned in a cranial direction with CT guideline attached to the skin at the axilla. We performed an intradermal injection in the periareolar area, using 4 ml of iopamidol with 1 ml of local anesthetic. The contrasted lymph route and SLN were identified in reconstructed three-dimensional imaging. The SLN spot was indicated by CT laser light navigator system. We established typical pattern of the lymphography: stain defect of SLN, stagnation of lymphatic route for preoperative diagnosis of metastatic SLN. SLN biopsy was performed using the fluorescence imaging system, Photodynamic Eye (pde-neo, Hamamatsu Photonics Co., Japan) referring to the point by axillary compression technique by plastic device. Intraoperative pathological analysis of SLN was examined, and an axillary lymph node dissection was performed in patients with SLN metastasis pathologically.

Results: The median age of the 350 patients was 59 (range 28 – 90) years old. One patient was male and others were female. CTLG were safely performed in all patients. CTLG could visualize lymphatic route and accurately identify SLN in 336 (96.0 %) and 343 (98.0 %) cases, respectively. Lymphatic routes of CTLG were completely consistent with those of fluorescence imaging. The mean number of SLN identified by CTLG was 1.1. Fifty of 350 patients had metastatic SLN pathologically, and 11 of them had micrometastases of SLNs. The accuracy for metastatic diagnosis of SLN using CTLG without micrometastasis was 84.1 %, sensitivity was 82.1 % and specificity was 84.3 %. The positive predictive value was 40.5 % and negative predictive value was 97.3 %. There were no complications associated with SLN identification.

Conclusion: CTLG in SLN biopsy has some advantages in that this method is simple and quite useful for obtaining accurate anatomic images of the SLN, lymph vessels, and tumor. CTLG could select the candidate with truly node negative cases in early breast cancer patients, because it predicts lymph node metastasis preoperatively from natural status of the lymphographic image.
**Title:** Real-time navigation for sentinel lymph node biopsy in breast cancer patients using projection mapping with indocyanine green fluorescence

Masahiro Takada¹, Megumi Takeuchi¹, Eiji Suzuki¹, Fumiaki Sato¹, Yoshiaki Matsumoto¹, Masae Torii¹, Nobuko Sakita-Kawaguchi¹, Yoshie Nakayama¹, Tomoko Okuda¹, Hiroto Nishino², Satoru Seo², Etsuro Hatano³ and Masakazu Toi¹.
¹Kyoto University Hospital, Kyoto, Japan; ²Kyoto University Hospital, Kyoto, Japan and ³Hyogo College of Medicine, Hyogo, Japan.

**Body:** Background
Sentinel lymph node (SLN) biopsy using indocyanine green fluorescence (fICG) method showed equal or better identification rate compared with blue dye or radioisotope (RI) method. In the fICG method, lymphatic vessels which drain into the SLNs can be seen through skin or subcutaneous tissue using near infrared camera (Photodynamic Eye®: PDE), and we can easily find the SLNs. However, whenever we observe the fluorescence images, we have to hold the PDE, turn off the operating light, and look at a monitor because fluorescence images cannot be seen directly. Medical imaging projection system (MIPS) is a new device which detects fluorescent emission from the organ and projects their images on the location of the fluorescence emission (Panasonic Connected Solutions Company, Japan). Projected images can be adjusted following the body movement or deformation of the organ. Therefore, MIPS could provide an option for real-time navigation for the SLN biopsy. The aim of this study was to evaluate the clinical utility of the MIPS.

**Patients and methods**
Patients with clinically node-negative primary breast cancer underwent the fICG SLN biopsy using MIPS. Primary endpoint was identification rate of the fICG method using MIPS. At first, the study was conducted as an interventional study because the MIPS was the unapproved medical device. After approval of the MIPS, this study was conducted as an observational study. The study protocol was approved by the institutional review board at Kyoto University Hospital. All patients provided informed consent to participate in this study.

**Results**
Between March 2016 and May 2017, 39 patients (40 procedures) underwent the fICG method SLN biopsy using MIPS. The median age was 55 years (range 32–74 years), and the median body mass index was 20.4 kg/m² (range 17.7–27.7 kg/m²). About half had tumor stage T1 (58%) and 8 (20.0%) had DCIS. 8 procedures (20%) were performed after preoperative systemic therapy (PST). As MIPS itself can illuminate the operating field, SLN biopsy using MIPS was successfully performed without operating light in all procedures. At least one SLN was detected using MIPS for all procedures and the identification rate was 100% (95% CI: 91–100%). Median number of SLNs detected by MIPS was 3 (range 1–9) for all procedures, and 3 (range 2–8) for procedures after PST. Two pathologically positive SLNs and one SLN which included isolated tumor cells were detected by MIPS. In 25 procedures, RI was also used. 62 of 97 SLNs detected by MIPS (64%) were also detected by RI. However, no SLNs were detected only by RI.

**Conclusions**
Although we still may not be able to avoid RI method because 25/40 (62.5%) procedures required the combined use of RI method, the fICG methods SLN biopsy using MIPS, which showed comparable identification rate of SLN with the conventional methods, could be useful tool with a view of allowing us to perform a real-time navigation surgery.

**Acknowledgements**
This study was supported by Acceleration Transformative research for Medical innovation, Japan Agency for Medical Research and Development (AMED).
Title: Predictors of axillary nodal metastasis based on gene expression and clinicopathological characteristics: Data from a population-based prospective study, the Sweden Cancerome Analysis Network – Breast (SCAN-B)

Looket Dihge¹, Johan Staaf², Johan Vallon-Christersson², Cecilia Hegardt², Jari Häkkinen², Åke Borg² and Lisa Rydén³. ¹Lund University; Skane University Hospital, Lund, Sweden; ²Lund University; Lund University Cancer Center; CREATE Health Strategic Centre for Translational Cancer Research, Lund, Sweden and ³Lund University; Skane University Hospital, Lund, Sweden.

Body: Background
Gene expression patterns show promise in estimating prognosis and directing adjuvant therapy, but its significance in guiding axillary treatment is sparsely evaluated. We aimed to identify predictors for nodal status based on gene expression patterns alongside clinicopathological characteristics, and to validate the performances as well as the prognostic importance of the predictors in a population-based context.

Material and Methods
The study assigned consecutive patients with primary breast cancer enrolled in the SCAN-B study (ClinicalTrials.gov ID: NCT02306096) in South Sweden between September 2010-March 2015. Exclusion criteria were: prior breast cancer, neoadjuvant therapy or unknown nodal status after surgical staging. Data on age, tumour size, multifocality, vascular invasion, NHG and ER/PR/HER2 status were retrieved. 3026 patients were successfully profiled by RNA sequencing (RNA-seq) forming the study analysis cohort. Patients enrolled during 2011 (n=1206) were excluded from predictor training/test sets and kept as an independent validation set. Seven machine-based learning algorithms were evaluated for all samples and for each of the molecular subtypes based on routine analysis: ER+/HER2-, HER2+ and TNBC. Primary outcome was discrimination (AUC) for N0/N+ based on either clinicopathological parameters, RNA-seq data or mixed data. Secondary outcome was to evaluate the prognostic value of the predictors. Kaplan-Meier estimates were used to portray univariate survival data in subgroups stratified by nodal status.

Results
The Swedish National Quality Registry for Breast Cancer revealed 5235 patients eligible for study inclusion, of which 89% were enrolled in the SCAN-B study (ClinicalTrials.gov ID: NCT02306096) in South Sweden between September 2010-March 2015. Exclusion criteria were: prior breast cancer, neoadjuvant therapy or unknown nodal status after surgical staging. Data on age, tumour size, multifocality, vascular invasion, NHG and ER/PR/HER2 status were retrieved. 3026 patients were successfully profiled by RNA sequencing (RNA-seq) forming the study analysis cohort. Patients enrolled during 2011 (n=1206) were excluded from predictor training/test sets and kept as an independent validation set. Seven machine-based learning algorithms were evaluated for all samples and for each of the molecular subtypes based on routine analysis: ER+/HER2-, HER2+ and TNBC. Primary outcome was discrimination (AUC) for N0/N+ based on either clinicopathological parameters, RNA-seq data or mixed data. Secondary outcome was to evaluate the prognostic value of the predictors. Kaplan-Meier estimates were used to portray univariate survival data in subgroups stratified by nodal status.

Conclusions
Subgroup-specific predictors for nodal status based on gene expression data alongside traditional clinicopathological characteristics were developed, and independently validated regarding performance and prognostic value, in a population-based breast cancer cohort. Integrating gene expression data in the preoperative setting may improve decision-making on the required extent of axillary surgery and systemic therapy needed.
Title: Association of quantitative MRI features with tumor infiltrating lymphocytes and treatment response prediction in HER2 positive subtype of breast cancer

Gaiane M Rauch1, Hongtu Zhu1, Heng Li1, Beatriz E Adrada1, Lumarie Santiago1, Rosalind P Candelaria1, Hao Wang1, Jessica Leung1, Jennifer Litton1, Yun Wu1, Rashmi Murthy1, Elizabeth A Mittendorf1 and Wei Yang1. 1The University of Texas MD Anderson Cancer Center, Houston, TX.

Objectives: To evaluate associations between qualitative and quantitative MRI features and tumor infiltrating lymphocytes (TIL) levels in HER2+ subtype of breast cancer, as potential prognostic non-invasive imaging markers for treatment response prediction.

Materials and Methods: Retrospective review of breast cancer patients who had MRI at staging, neoadjuvant chemotherapy and surgery from January 1, 2008 through December 31, 2015 was performed. BI-RADS lexicon was used for lesion evaluation. Demographic, imaging, and pathologic data including TIL levels were documented. Quantitative MRI texture analysis was performed using 3 types of textural features (TF): local binary patterns (LBP), gray-level co-occurrence matrix (GLCM), and threshold adjacency statistics (TAS). Associations between MRI quantitative TF, TIL levels, and pathologic complete response (pCR) were evaluated by Pearson correlation and logistic regression.

Results: There were 50 HER2+ patients (median age 51 years, range 29-59) with pretreatment MRI and TIL status for analysis; 27 patients had pCR at surgery. Qualitative MRI analysis showed that mass rim-enhancement (p<0.05) and fast early enhancement kinetics (p<0.01) were associated with higher TIL levels. No association between qualitative MRI features and pCR was found. Nine TF significantly correlated with pCR (p<0.05): angular 2nd moment (GLCM), 75 percentile (LBP), standard deviation (GLCM), adjacency 0-5 (TAS). This is indicative of association of tumor heterogeneity with pCR. Three TF were significantly associated with high TIL levels (p<0.05): standard deviation, adjacency 1 and 2. Additional four TF had high association with TIL (p<0.1): sum entropy, adjacency 0, 3 and 4. These findings showed that increased heterogeneity, complexity and entropy were associated with high TIL level. Three TF were significantly associated with both, pCR and TIL (p<0.05): 75 percentile, standard deviation, adjacency 8.

Conclusion: Quantitative tumor texture analysis based on statistical modeling showed specific nine TF indicative of tumor heterogeneity associated with pCR; and seven TF indicative of increased heterogeneity, complexity, and entropy associated with high TIL levels in HER2+ breast cancer. Analysis of associations of MRI quantitative TF with pCR and TIL in HER2+ breast cancer may help to develop prognostic non-invasive imaging markers for treatment response prediction.
2017 San Antonio Breast Cancer Symposium

Publication Number: PD2-10

Title: Radiomic phenotypes of tumor heterogeneity from pre-operative DCE-MRI independently predict breast cancer recurrence after 10-year follow-up from primary invasive diagnosis

Rhea D Chitalia¹, Jennifer Rowland¹, Elizabeth McDonald¹, Lauren Pantalone¹, Eric Cohen¹, Aimilia Gastounioti¹, Kathleen Thomas¹, Rebecca Batiste², Michael Feldman², Mitchell D Schnall¹, Emily F Conant¹ and Despina Kontos¹. ¹University of Pennsylvania, Philadelphia, PA and ²Department of Pathology and Laboratory Medicine, Philadelphia, PA.

Body: Purpose: To investigate whether pre-operative MRI phenotypes of tumor heterogeneity have independent prognostic value and determine their associations with recurrence-free-survival (RFS) using 10-year follow-up after primary invasive breast cancer diagnosis.

Materials and Methods: The DCE-MRI images of 94 women diagnosed with primary invasive breast cancer, who had complete histopathologic and 10-year follow up data available, were chosen from a previously conducted multimodality clinical trial cohort (2002-2006). For each woman, the signal enhancement ratio (SER) map was calculated for the most representative slice of the primary lesion. Radiomic features (including first order histogram, run-length, structural, and co-occurrence matrix features) and morphologic measures (perimeter, area, ellipticity, and convexity) were extracted and summarized over four quadrants of the tumor. To identify intrinsic phenotypes of tumor heterogeneity, unsupervised hierarchical clustering was applied to the extracted feature vectors after z-score normalization, where cluster cutoffs were determined using Consensus Clustering and the SigClust method. The normalized feature vectors were further averaged to generate a composite heterogeneity score for each tumor. Differences across phenotypes by recurrence, established prognostic factors (ER, PR, HER2, Clinical Stage, Ki67%), and heterogeneity score were assessed using Chi-square and Kruskal-Wallis tests. Kaplan-Meier curves were used to display survival probabilities across phenotypes, adjusting for time-to-event data. Heterogeneity phenotype assignments were added to a baseline Cox proportional hazards model with established prognostic factors to predict RFS. The log-likelihood test was used to assess goodness-of-fit and model discriminative capacity was evaluated using the c-statistic.

Results: Our sample included 14 recurrences (15%). Unsupervised clustering identified three intrinsic phenotypes that ranged from low to high heterogeneity (p<0.001). The most heterogeneous phenotype contained all recurrences (p<0.001). Clinical stage was also different among phenotypes (p=0.05). The augmented model incorporating heterogeneity phenotypes had higher discriminatory capacity (c-statistic=0.86), compared to the baseline model including only established histopathologic prognostic factors (c-statistic=0.79).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline Model Hazard Ratio</th>
<th>Baseline Model + MRI Tumor Heterogeneity Phenotype* Hazard Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrogen Receptor (ER)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Progesterone Receptor (PR)</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>HER2 Receptor (Her2)</td>
<td>0.65</td>
<td>0.72</td>
</tr>
<tr>
<td>Ki67 (%)</td>
<td>1.00</td>
<td>0.99</td>
</tr>
<tr>
<td>Clinical Stage</td>
<td>2.55</td>
<td>1.94</td>
</tr>
<tr>
<td>Heterogeneity phenotype</td>
<td>p&lt;0.001</td>
<td></td>
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</tbody>
</table>

*Log-likelihood ratio between models p-value <0.001

Conclusion: Intrinsic phenotypes of tumor heterogeneity in pre-operative DCE-MRI of primary invasive breast cancer may provide additional prognostic information to established histopathologic factors. Independent validation is needed to determine phenotype reproducibility and their independent associations to RFS when also including additional molecular/histopathologic prognostic factors.
Contrast-enhanced spectral mammography is comparable to MRI in the assessment of residual breast cancer following neoadjuvant systemic therapy

Talal Hilal¹, Matthew Covington¹, Mark Sugi¹, Nan Zhang¹, Barbara Pockaj¹, Donald Northfelt¹, Idris T Ocal¹ and Bhavika K Patel¹.
¹Mayo Clinic, Phoenix, AZ.

OBJECTIVE: Currently, no study has assessed the performance of contrast-enhanced spectral mammography (CESM) in evaluating tumor response in breast cancer patients undergoing neoadjuvant systemic therapy (NST). This study aims to evaluate whether the accuracy of CESM is comparable to MRI in detection of residual breast cancer following NST.

MATERIALS AND METHODS: Retrospective review of CESM cases at our institution between September 2014 and June 2016 identified patients who had both CESM and MRI pre- and post-NST with pathologic assessment after surgical management. Size of residual malignancy (if any) on post-neoadjuvant CESM and MRI was compared to surgical pathology (reference standard). Pathologic complete response (pCR) was documented and compared to Residual Cancer Burden (RCB) score for confirmation. Bland-Altman plots were used to visualize the differences between CESM/MRI and pathologic tumor size.

RESULTS: Forty female patients met inclusion criteria. Mean age was 52.3 years (range 35-73). Type of NST included: 34 (85%) chemotherapy and 6 (15%) endocrine therapy. Histological analysis showed invasive ductal carcinoma in 38 (95%), the remaining cases consisted of one invasive lobular carcinoma, and one mixed invasive carcinoma. Mean tumor size after NST was 10.3 mm (range 0-75 mm) for CESM and 9.7 mm (range 0-60 mm) for MRI compared to 15.7 mm (range 0-100 mm) on final surgical pathology. Equivalence tests demonstrated that the mean tumor size measured by CESM or by MRI is equivalent to the mean tumor size measured by pathology within -1 and 1 cm range (p=0.0132 for CESM and p=0.0194 for MRI).

A complete radiologic response was seen in 25 CESM and 22 MRI cases which was confirmed by pathology in 17 and 14, respectively. Alternatively, CESM and MRI demonstrated residual disease in 15 patients and 18 patients respectively and this was confirmed on pathology in 15 and 15, respectively.

Accuracy of CESM vs. MRI

<table>
<thead>
<tr>
<th>Modality</th>
<th>Residual Disease by Pathology (N=23)</th>
<th>Complete Response by Pathology (N=17)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residual Disease by CESM (N=15)</td>
<td>15</td>
<td>0</td>
<td>65.2%</td>
<td>100%</td>
<td>100%</td>
<td>68%</td>
</tr>
<tr>
<td>Complete Response by CESM (N=25)</td>
<td>8</td>
<td>17</td>
<td>65.2%</td>
<td>82.4%</td>
<td>83.3%</td>
<td>63.6%</td>
</tr>
<tr>
<td>Residual Disease by MRI (N=18)</td>
<td>15</td>
<td>3</td>
<td>65.2%</td>
<td>82.4%</td>
<td>83.3%</td>
<td>63.6%</td>
</tr>
<tr>
<td>Complete Response by MRI (N=22)</td>
<td>8</td>
<td>14</td>
<td>65.2%</td>
<td>82.4%</td>
<td>83.3%</td>
<td>63.6%</td>
</tr>
</tbody>
</table>
All patients who achieved a pCR had an RCB score of 0 indicating no residual cancer in lymph nodes. Among patients with residual disease, their mean RCB score was 2.6 (range 0.8-4.18).

CONCLUSION: In this study, CESM was comparable to MRI in assessing residual malignancy after completion of NST, thereby offering a potentially faster and less expensive alternative to MRI for monitoring treatment response in the neoadjuvant setting.
Palbociclib plus letrozole has improved both progression free survival and overall response rate in metastatic breast cancer (MBC) patients. For response to palbociclib, the best biomarker is ER expression. $^{16}\alpha^{-}[^{18}\text{F}]\text{Fluoro-17}\beta\text{-estradiol (FES)}$-PET allows whole body ER level assessment, and provides insight in the heterogeneity of ER expression throughout the body. We hypothesized that lesions with low uptake on FES-PET are unlikely to respond to letrozole plus palbociclib. METHODS: Post-menopausal women with ER positive MBC were eligible for this pilot study. All patients were staged with fludeoxyglucose (FDG)-PET and CT scan, and in addition a FES-PET was performed at baseline. After 8 weeks treatment an FDG-PET/CT was used for response evaluation. The primary endpoint was the relation between standard uptake value (SUV) per lesion on FES-PET to response, as measured by RECIST 1.1 criteria in case of measurable disease. In case of non-measurable bone lesions, progression was defined as an increase in SUV on FDG-PET of more than 30% per lesion compared to baseline (based on PERCIST). RESULTS: 15 patients were included of which 14 were evaluable for primary endpoint. Mean age was 50 years (range 35-76). Median number of prior therapies was 1. A total of 280 lesions were detected on conventional imaging of which 50 showed low uptake (SUV<1.5) on FES-PET. 29/50 low uptake lesions showed progression based on diagnostic CT (n=9) or FDG PET (n=20). In contrast, 28/230 FES positive lesions showed progression.

<table>
<thead>
<tr>
<th></th>
<th>Response</th>
<th>Stable disease</th>
<th>Progression</th>
</tr>
</thead>
<tbody>
<tr>
<td>FES uptake &gt;1.5</td>
<td>108</td>
<td>94</td>
<td>28</td>
</tr>
<tr>
<td>FES uptake &lt;1.5</td>
<td>7</td>
<td>14</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>115</td>
<td>108</td>
<td>57</td>
</tr>
</tbody>
</table>

DISCUSSION: this pilot trial indicates negative predictive value of low FES uptake for response to letrozol plus palbociclib in ER positive MBC. The FES-PET may therefore be a biomarker for response for this combination treatment. This will have to be explored further in future clinical trials.
Title: Benefits to breast screening beyond mortality reduction

Kenneth J Elder¹, Carolyn Nickson², Samuel Cooke², Dorothy Machalek², Alison Rose¹, Arlene Mou¹, John P Collins¹, Allan Park¹, Richard De Boer¹, Claire Phillips³, Vicki Pridmore⁴, Helen Farrugia⁵ and Gregory B Mann¹²³. ¹Royal Women's Hospital, Melbourne, Victoria, Australia; ²University of Melbourne; ³The Royal Melbourne Hospital; ⁴BreastScreen Victoria and ⁵Victorian Cancer Registry.

Body: Background
The value of population-based mammographic screening has been questioned by those who believe that the reduction in mortality from earlier diagnosis is outweighed by harms including overdiagnosis and overtreatment. Much of these commentaries assume that all Early-Stage Breast Cancer (ESBC) is treated the same way after diagnosis; with extensive therapies including surgery, radiotherapy and chemotherapy being standard. Intensity of treatment received is rarely mentioned in the debate. We hypothesised that those diagnosed through a screening program (Active Screeners (AS)) would receive less extensive surgical treatment and less intense adjuvant therapies than those not recently screened (NRS). If demonstrated, these differences would form an important component of the debate over the role of mammographic screening.

Methods
Retrospective analysis of a consecutive cohort of female patients aged 50-69 and managed for ESBC (invasive or DCIS) during 2007-2013 within a large metropolitan Breast Service, diagnosed either via a population screening program (AS) or outside of the program (NRS). Data on patient characteristics, symptoms, mode of detection, tumour pathology, surgical intervention and adjuvant treatment recommendations were derived from prospectively collected Multi-Disciplinary Meeting (MDM) records. Patients with metastatic disease or prior treatment for breast cancer were excluded.

Results
791 cases were identified (569 with screen-detected cancer, 53 with interval cancers and 169 cancers diagnosed in women not recently screened). Invasive cancers in the AS group were much smaller than in the NRS group – mean 17mm versus 26mm. The AS group had lower grade invasive cancer – grade 1, 2 and 3 were 27%, 42%, 31% - compared with 10%, 39% and 52% in the NRS group. The AS group were more likely to have ER+ve cancers (88% vs 80%) and less likely to have nodal involvement (26% vs 48%). For invasive breast cancer, the NRS group were more than twice as likely to undergo mastectomy than cancers in the AS group (35% vs 16%). Axillary dissections were more common in the NRS than the AS group (43% vs 19%). Adjuvant chemotherapy was recommended more frequently for the NRS group compared to the AS group (65% vs 37%), as was post-mastectomy radiotherapy (58% vs 39%). Endocrine therapy was less often recommended to the NRS group (86% versus 77%).

Conclusion
Women diagnosed with early stage breast cancer who are participating in a population based screening program are less likely to receive mastectomy and/or axillary dissection, less likely to receive adjuvant chemotherapy and less likely to receive post-mastectomy radiotherapy. These differences in treatment intensity should be considered in the debate surrounding mammographic screening.
Title: Participation in a personalized breast cancer screening trial does not increase anxiety at baseline

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Body: Introduction: The purpose of this study is to examine whether participation in a personalized screening trial is associated with anxiety or breast cancer worry. The Patient Centered Outcomes Research Institute recently funded WISDOM (Women Informed to Screen Depending On Measures of risk), which is a randomized trial that tests the safety and efficacy of basing starting age, stopping age, frequency and modality of breast cancer screening on individual risk (Clinical Trials Identifier NCT02620852).

Methods: In WISDOM, participants can be randomized to annual screening or personalized screening arm, or self-select an arm an observational cohort. This interim analysis examined the first 1817 participants to determine if the personalized risk arm is acceptable and to explore whether baseline anxiety was associated with study arm. For acceptability our target was to have >60% of participants agree to randomization. Participants completed questions about their Risk Perception, the PROMIS Anxiety short form 8a (total scores 8-40 with higher scores indicating more anxiety), and Breast Cancer Risk Worry (BCRW) survey (total scores 5-20) with higher scores indicating more worry) at baseline and before they were given information on their personal risk or study assignment. For the purposes of these analyses, we defined high anxiety to be the percentage of participants scoring =>22 on the PROMIS and >8 on the BCRW.

Results: The participants were recruited from three sites (UCSD, UCSF, Sanford Health). Of the 1817 initial participants, 1643 completed the baseline questionnaire. Participants has a mean age of 57 years (SD 9). 15.8% felt their chances of developing breast cancer was high, 19.5% felt their chance of developing breast cancer was greater than the average women, and 56.6% felt their lifetime risk of developing breast cancer was >25. Risk perception was not significantly different between women who opted to be randomized versus the observational arm.

The majority of participants were willing to be randomly assigned to an arm (1071/1643, 65.1%). Of those who joined the observational cohort, the majority selected personalized risk arm (474/572, 82.9%). Overall, PROMIS anxiety scores were low at baseline (14.0 MEAN (SD 4.6)) as were the Breast Cancer Risk Worry scores (5.7 MEAN (SD 1.05)). Less than 8% of participants had PROMIS scores >22 and that did not vary across the randomized or observational groups (P=0.2)). About 2% of participants had a BCRW scores >8. Women who worried with breast cancer were more likely to select to be in the observational (3.5%) than randomized (1.7%) arm of the study (P=0.02).

Conclusions: For the women approached to participate in Wisdom, personalized screening was acceptable alternative to annual mammography. Participants in general overestimated their lifetime risk of breast cancer, had very low anxiety and low breast cancer worry. Those who were worried about breast cancer opted more often for the observational arm of the study to allow them to choose between the personalized versus annual arm. Future analyses will follow participants prospectively to determine adherence to assigned or selected arm, and whether anxiety changes after receipt of their personalized risk information.
2017 San Antonio Breast Cancer Symposium

Publication Number: PD2-15

Title: Effect of mammography screening frequency on false-positive biopsy rates and detection of local recurrence among breast cancer survivors

Sarah Yuan¹, Haley J Manley¹, Richard Ha¹, Anqi Yu¹, Jeanine M Genkinger¹ and Katherine D Crew¹. ¹Columbia University Medical Center, New York, NY.

Body: Introduction: Current guidelines are for yearly mammograms in women with early-stage breast cancer. Among breast cancer survivors treated with lumpectomy, semi-annual compared to annual screening mammography of the ipsilateral breast has been associated with early detection of local recurrence. However, a potential harm of more frequent screening is false-positive breast biopsies that may lead to negative psychosocial effects and increased costs. Our objective was to investigate how frequency of screening mammograms affects rates of false-positive biopsy results and local recurrences among breast cancer survivors.

Methods: We conducted a retrospective cohort study at Columbia University Medical Center (CUMC) in New York, NY of women diagnosed with stage 0-III breast cancer between 2007 and 2015, who were treated with lumpectomy and had at least 2 screening mammograms at CUMC within the first 3 years after diagnosis. Demographic and clinical information, including tumor characteristics and breast cancer treatments, were collected from the electronic health record. Frequency of mammography screening was defined as the median interval between 2 consecutive mammograms (every 6 months vs. yearly). Both false-positive biopsy results and local recurrences were identified by review of breast pathology reports. A false-positive biopsy was defined as a diagnostic breast biopsy without evidence of invasive or non-invasive cancer. Descriptive statistics and logistic regression models were conducted to examine relationships between covariates and either false-positive biopsy or local recurrence.

Results: In our sample (n=1257), the median age at breast cancer diagnosis was 60 years (range, 24-93), including 47% non-Hispanic white, 14% non-Hispanic black, 31% Hispanic, and 7% Asian. Nearly 80% of women had semi-annual screening mammography of the ipsilateral breast during the first 3 years after breast cancer diagnosis. In univariate analysis, higher body mass index, more advanced stage disease, higher tumor grade, and receipt of chemotherapy, hormonal therapy, and radiation therapy were associated with more frequent screening. Comparing women who screened every 6 months vs. yearly, there was no difference in local recurrence rates (4.1% vs. 3.9%), including screen-detected or invasive/non-invasive breast cancer recurrences. In multivariable analysis, women who screened every 6 months compared to yearly had a greater than 2-fold increased risk of having a false-positive biopsy (OR: 2.40; 95% CI: 1.50-3.86). Also, younger age at diagnosis, higher tumor grade, and receipt of chemotherapy were associated with higher false positive rates, adjusting for covariates.

Conclusions: We observed that women with early-stage breast cancer treated with lumpectomy who underwent semi-annual vs. annual screening mammography had more false-positive breast biopsies, but no difference in local recurrence rates. To date, there is no evidence that more frequent screening in breast cancer patients is associated with improved survival. Future studies are needed to determine optimal screening strategies for breast cancer survivors, including frequency of screening and use of supplemental breast imaging with ultrasound, MRI, or tomosynthesis.
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**Body: Background**
Breast cancer screening by mammography has been shown to reduce breast cancer mortality, however false positive recall rates have consistently been identified as a harm of organized screening. The extent to which these recalled women are at increased risk of future breast cancer remains unclear.

**Methods**
The British Columbia Cancer Agency Screening Mammography Program (SMP), the first organized breast screening program in Canada offers screening mammography for women aged 40-74 since 1988. All women who had two or more screening mammograms between 1988-2013 within the SMP were included in the study. They were followed until a breast cancer diagnosis, last screen date +5 years, or end of follow-up on Dec 31, 2013, whichever came first. The relative risk (RR) of breast cancer for women with a false-positive test compared with women with negative tests was estimated with Poisson regression, adjusted for age, and five calendar periods.

**Results**
A total of 772,289 women with 4.82 million screening mammograms and a median follow up of 11.8 years were included. There were 238,860 women with false positive findings and 26,950 cancers of which 16,084 screen detected and 10,866 non screen detected. Women without any false positive mammogram had a cancer incidence rate of 245/100 000 person-years at risk, whereas women with a false-positive test had an absolute rate of 447/100 000 person-years at risk. The adjusted RR [Value (95% CI)] of breast cancer after the first false-positive test was 1.73(1.68-1.77) for all, 1.65(1.61-1.70) for invasive, and 2.13(2.01-2.27) for in situ cancers respectively. The RR remained increased beyond 8 years after the false-positive test. Of the 5157 screen detected cancers after the first false positive test, 3358 (65%) were on the ipsilateral breast while 1799 (35%) were on the contralateral breast. Women with only one, two, three or four+ false positive test(s) had RR of 1.88(1.83-1.93), 1.42(1.35-1.49), 1.17(1.05-1.30), and 1.08(0.87-1.34) respectively for all cancers. Women with breast density >50% at the time of false positive test had a twofold risk of breast cancer with a RR of 2.07(1.99-2.14), while those with breast density <50% had a RR of 1.58(1.54-1.63). When stratified for mammographic features found on the first false positive mammogram, architectural distortion plus mass had the highest RR 4.68(3.16-6.93) for invasive cancers while calcifications alone and calcifications plus asymmetry had highest RR 5.57(4.88-6.36) and 4.07(2.49-6.66) for in situ cancers.

**Conclusion**
False positive mammogram correlates with an increased risk of developing breast cancer. 65% of the screen detected breast cancers post false positive mammogram occur in the ipsilateral breast. Mammographic abnormality features of the false positive mammogram are found to be predictors for the type of future breast cancer. Mammographic features at the time of recall predicts risk of subsequent cancer and may warrant increased surveillance.
Title: Dual energy: Potentialities of contrast enhanced mammography in breast disease diagnosis and follow-up

Elsa Cossu¹, Elisa Costano¹, Roberta Di Trapano¹, Maria Claudia Pensabene¹, Carla Di Stefano¹, Stefania Fosi¹, Valeria Fiaschetti¹, Ilaria Portarena¹ and Oreste Buonomo¹. ¹PTV Foundation, University of Rome Tor Vergata, Roma, Rome, Italy.

Body: Purpose: Contrast-enhanced Dual-Energy digital mammography (DE) is a recently used investigation that consists in acquiring low and high energy images after intravenous injection of iodinated contrast medium. The purpose of this study is to describe our experience about the role of DE in the diagnosis and follow-up of breast cancer.

Materials and Methods: 50 patients, with heterogeneous breast pattern, already studied by mammography and ultrasound, were selected. They underwent DE mammography: 17 for mammographic or ultrasonographic suspicious findings (9/17 also examined with MRI), 21 for loco-regional staging (21/21 studied with MRI), 10 for oncologic follow-up (6/10 also underwent MRI). DE has been interrupted in two cases due to technical problems. Exclusion criteria of the study were: breast implants, pregnancy or lactation and contraindications to administration of contrast medium. DE results were compared with the post-operative or post-bioplastic (Tru-cut or Vacuum Assisted Biopsy) histological findings.

Results: DE showed a sensitivity of 76% in evaluation of Patients with mammographic or ultrasonographic suspicious findings: in comparison with MRI (9 patients), DE had a 55% specificity (vs 22% of MRI). In locoregional staging DE proved to be more accurate than MRI in dimensional evaluation of unifocal cancer: DE accuracy was 91% vs 62% of MRI, which overestimated the size of most of lesions. In the count of tumor foci number in case of multifocal/multicentric disease MRI corresponded in 9/11 cases with histology (vs 3/11 cases of DE). DE showed a sensitivity of 100% and a 85.7% specificity in assessing oncologic follow-up Patients: in the comparison between DE and MRI (6 patients), DE got 1 false positive result, while MRI 3.

Conclusion: DE can be a valuable diagnostic tool to help conventional techniques; it is also a possible alternative to MRI in some selected type of patients or where MRI is not available.
Title: Immune profile of small HER2+ tumors in the APT trial

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Body: Background: APT is a single arm multicenter, phase II study of paclitaxel and trastuzumab. Patients with HER2+ breast cancer with negative nodes and tumor size ≤ 3 cm were eligible. Disease-free survival at 7 years was 93.3% with only 4 distant recurrences. Characterizing intrinsic subtype and immune profiles of these smaller tumors may help us better understand if the biology of smaller HER2+ tumors is different than larger tumors that have been previously characterized.

Methods: Intrinsic subtyping by PAM50 and immune signatures by PanCancer Immune Profiling Panel were performed on the nCounter Analysis system on archival tissue. Tissue was also tested by immunohistochemistry for PDL1 (tumoral and immune cells) and was assessed for tumor infiltrating lymphocytes (TILs) using guidelines from the International TILs Working Group. TILS were categorized as follows: low (≤10%), intermediate (10-60%), high (≥60%); PD-L1 was characterized as low (0-1%), intermediate (1-10%), and high (>10%).

Results: PAM50 data were available for 209 of the 406 cases: 142 (68%) were HER2-Enriched (HER2E), 22 (11%) Luminal A, 25 (12%) Luminal B, and 20 (10%) Basal-like. Immune profile information was available for 162 cases, of which 138 also had intrinsic subtype data. 184 cases were evaluated for PD-L1, and 210 cases for TILs. There was a strong correlation between PD-L1 in the tumor and lymphoid compartments (McNemar's chi-squared, p= 6.5x10⁻¹²). High tumoral PD-L1 was seen with higher frequency in the HER2E subset, while high immune cell PD-L1 and high TILs were seen with higher frequency within both the HER2E and Basal-like subtypes (Table). Immune profile data demonstrates that the T-cell signature has the strongest association with TILs, and that luminal A tumors tend to have very similar immune profiles while there is more variable expression of immune cell types with the basal-like tumors. Further work is ongoing to complete PAM50 and intrinsic subtype profiling of remaining tumors, and additional relationships between immune profile and intrinsic subtype will be presented at the meeting.

Conclusions: The majority of small HER2+ breast cancers are HER2E, and tumors of this intrinsic subtype have the highest prevalence of high expression of PDL1 and high TILs, suggesting that these tumors may be more immunogenic than the luminal A and B HER2+ tumors. Further work to explore immune profile data by intrinsic subtype is ongoing and may have implications for future trial design.

<table>
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<th>Luminal A</th>
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<th>Basal-Like</th>
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<td>High</td>
<td>8</td>
<td>1</td>
<td>0</td>
<td>0</td>
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<tr>
<td>PDL1 (immune cell)</td>
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<td></td>
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<tr>
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<td>11</td>
<td>3</td>
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<tr>
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<td>5</td>
<td>4</td>
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<tr>
<td>High</td>
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<td>1</td>
<td>6</td>
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<td>TILs</td>
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<tr>
<td>Low</td>
<td>64</td>
<td>15</td>
<td>16</td>
<td>5</td>
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<tr>
<td>Intermediate</td>
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<td>3</td>
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<tr>
<td>High</td>
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Title: Comparison of HER2, estrogen and progesterone receptor expression profiles of primary tumor, synchronous axillary lymph node metastases and circulating tumor cells in early breast cancer patients

Bahriye Aktas1,2, Laura Weydandt1,2, Daniela Westerwick3, Fabian Mairinger3, Sabine Kasimir-Bauer1, Rainer Kimmig1, Kurt Schmid3 and Agnes Bankfalvi3. 1University Hospital Essen; 2University Hospital Leipzig and 3University Hospital Essen.

Body: Background: Targeted systemic therapy in early breast cancer with synchronous lymph node metastasis is currently based on the expression of the hormone receptors (ER/PR ≥1%) and overexpression of HER2 in the primary tumor. However, the expression of these predictive markers on lymph node metastases as well as on circulating tumor cells (CTCs), the precursors of metastatic disease, has not yet been taken into account. The aim of the present study was to compare the HER2/ER/PR expression profiles of primary tumors, synchronous lymph node metastases and circulating tumor cells in early breast cancer patients and relate the results to 5-year overall (OS) and disease-free survival (DFS).

Patients and Methods: 76 patients with early breast cancer diagnosed between 2006 and 2010 were enrolled in this study. Blood was obtained at the time of first diagnosis of disease and analyzed for CTCs using the AdnaTest BreastCancer (Qiagen Hannover GmbH) for the expression of EpCAM, MUC-1, HER2, ER and PR respectively. Formalin-fixed and paraffin-embedded archival tissues of the primary tumors and the lymph node metastases were analyzed by two pathologists. ER, PR and HER2 expression was assessed by fully-automated immunohistochemistry (Ventana medical Systems, USA) and HER2/CEN17 dual chromogenic in situ hybridization (Zytomed Systems, Germany) according to modified ASCO/CAP guidelines (2010 and 2013).

Results: 2006-2010 the detection rate for CTCs was 22% (17/76 patients). In the primary tumors, the expression rate of HER2 was 16% (13/76 patients), 78% for ER (61/76 patients) and 75% for PR (57/76 patients) respectively. Changes in biomarker profiles between primary tumors, metastases and CTCs, as a whole, were observed in 89% of the cases (68/76). The discordance rates between primary tumors and lymph node metastases were 10% for HER2 (p<0.001), 5% for ER (p=p<0.001) and 11% for PR (p<0.001). The intrinsic subtypes between primary tumors and lymph node metastases changed in 16% of all cases (12/76 patients; gain of HER2 in four, activation of HRs in three cases, loss of HER2 in three and loss of HRs in one case becoming triple negative). Of note, both OS and DFS of patients with subtype discordance were reduced to a median of 41 months (mean:39) vs 56 months (mean:51).

CTCs were either triple negative or HER2 positive. Discordance rates for HER2, ER and PR status compared to the primary tumors were 16% (12/76 patients), 83% (63/76 patients) and 72% (55/76 patients), respectively. Discordant rates between lymph node metastases and CTCs were 25% for HER2 (19/76 patients), 82% for ER (62/76 patients) and 72% for PR (55/76 patients). Enlarged results including the cohort 2010-2012 will be available for SABCS 2017.

Conclusion: Our preliminarily results demonstrate, that changes in molecular profiles are the rule rather than the exception throughout tumor progression in breast cancer. In cases with discordant biomarker profiles, prognosis seems to follow the subtype of lymph node metastasis. Biomarker or subtype shift may be of essential therapeutic significance for individual patients.
Circulating tumor DNA in HER2 amplified breast cancer: A translational research substudy of the NeoALTTO phase 3 trial

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Body: Purpose. To evaluate whether circulating tumor DNA (ctDNA) was associated with response to neoadjuvant anti-HER2 targeted therapies (NAT) in patients enrolled in the Neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimization (NeoALTTO) trial (BIG 1-06).

Methods. Cell-free DNA from plasma collected before NAT, at week 2 and before surgery were processed using patient-specific droplet digital polymerase chain reaction (ddPCR) assays for the detection of PIK3CA and p53 mutations. These mutations had been previously detected in baseline primary tumor biopsy samples using mass spectrometry-based genotyping, exome or RNA sequencing. Chi-square test was used to evaluate differences in ctDNA detection according to the NeoALTTO stratification factors: clinical tumor size (T2 vs T3/4), clinical nodal status (N0/1 vs N2), Hormone Receptor status, HR (positive vs negative) and type of breast surgery planned (conservative vs mastectomy). Associations between ctDNA detection and either pathological complete response (pCR) or event-free survival (EFS) after adjustment for the NeoALTTO stratification factors were investigated using logistic and Cox regression models, respectively.

Results. A total of 69 of 455 (15.2%) patients enrolled in the NeoALTTO trial had either PIK3CA (55 patients) or p53 (14 patients) mutations detected in the baseline tumor samples and at least one plasma sample with evaluable ctDNA results. Out of the 69 patients, 36 (52.2%) had HR-negative disease, 45 (65.2%) had T2 tumors, 55 (79.7%) N0/1 disease, 50 (72.5%) were candidates for mastectomy and 27 (39.1%) received trastuzumab and lapatinib in combination. ctDNA before NAT, at week 2 and before surgery was detected in 29 (42%) of 69, in 13 of 64 patients (20.3%) and in 4 of 59 (6.7%) patients analyzed, respectively. There was no significant difference in ctDNA detection before NAT according to HR status, clinical tumor size, nodal status or type of planned breast surgery. After adjustment for the NeoALTTO stratification factors, ctDNA detection before NAT was associated with decreased odds of achieving pCR, (odds ratio, OR 0.16, 95% CI 0.04-0.71, p=0.016), but not with EFS (Hazard Ratio 1.32, 95%CI 0.74-2.37, p=0.345). Neither ctDNA detection at week 2 nor before surgery was significantly associated with either pCR or EFS but these analyses were underpowered.

Conclusion. In patients with HER2-amplified tumors and either PIK3CA or p53 mutations, detection of ctDNA before neoadjuvant anti-HER2 targeted therapies is associated with decreased probability of pCR.
2017 San Antonio Breast Cancer Symposium

Publication Number: PD3-04

Title: Complete pathologic response rate to neoadjuvant chemotherapy increases with increasing HER2 ratio in HER2 over-expressing breast cancer: Analysis of the National cancer database (NCDB)

Kathriena Greenwell¹, Lala Hussain², Ching Ho¹, Erik Dunki-Jacobs¹, David Lee¹, Matthew Bramlage³, Gordon Bills³, Apurva Mehta¹, Jason Jones¹, Amie Jackson¹ and Barbara Wexelman¹. ¹Trihealth Cancer Institute, Cincinnati, OH; ²Trihealth Hatton Research Institute, Cincinnati, OH and ³Trihealth, Cincinnati, OH.

Body: Background: HER2-positive (HER2+) breast cancer is an aggressive subtype that overexpresses human epidermal growth factor receptor 2 promoting cancer cell growth. Monoclonal antibodies targeting the HER2 receptor have improved survival for this patient population, and current NCCN guidelines recommend consideration of neoadjuvant anti-HER2 therapy (NAC) in Stage 2 & 3 HER2+ breast cancer. Pathologic complete response (pCR) to NAC has correlated with longer disease free survival in multiple trials.

Per ASCO-CAP guidelines tumors are considered HER2+ if HER2 copy number ≥ 6/cell, HER2/CEP17 ratio ≥ 2, or ratio<2 & HER2 copy number ≥6/cell. We hypothesize that patients with higher HER2 ratios will have higher rates of pCR after NAC.

Methods: The National Cancer Database is supported by the American College of Surgeons and the American Cancer Society containing de-identified patient treatment data from over 1,500 US facilities. We performed a retrospective review comparing pCR rates after NAC based on HER2 ratio. Patients were excluded if they were HER2 negative, did not undergo NAC, or if the HER2 ratio was not recorded. Chi-squared and Fisher’s exact test were used to compare pCR versus partial response between deciles of HER2 ratios.

Results: The NCDB included 237,118 patients with HER2 equivocal or HER2+ breast tumors. 29,291 of these patients underwent NAC, and HER2 ratios were recorded in 14,597 of the NAC cases. The majority (98%) of included cases were from 2010-2014. A pCR was noted in 9,752 patients and 11,402 patients had a partial response. No response was observed in 1,735 patients and 6,402 patients had a response but the degree was not recorded.

HER2 ratios were significantly different between pCR vs. partial response groups, p <0.001. We identified a direct relationship between increasing HER2 ratio and response to NAC. For ratios 2-2.9, 23.6% achieved pCR and 44.7% had a partial response. For ratio of 5-5.9, 40.7% achieved pCR and even higher rates of pCR were noted for ratios 8-8.9: 49.5% achieved pCR. While both estrogen receptor (ER) positive and ER negative tumors demonstrated this trend, ER negative tumors had higher rates of pCR (ER negative pCR range 37.6% to 59.4% vs ER positive pCR range 16.9% to 42.3%, p<0.01).

Conclusion: Contrary to current dogma, not all HER2+ tumors respond similarly to NAC. We demonstrate a linear relationship between HER2 ratio and pCR in over 14,000 patients. Those with HER2 ratios ≥5.0 were more likely to achieve pCR compared to patients with ratio ≤4.9. The NCDB reflects current clinical practice across the country not restricted to confines of clinical trials, and in this population higher HER2 ratios are predictive of pCR after NAC.

Response to NAC by Her2 Ratio- Complete vs Partial Response

<table>
<thead>
<tr>
<th>HER2 Ratio</th>
<th>Response to NAC</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Complete Response- pCR (N)</td>
<td>Partial Response (N)</td>
</tr>
<tr>
<td>1.00- 1.99</td>
<td>1418</td>
<td>19.5%</td>
</tr>
<tr>
<td>2.00- 2.99</td>
<td>514</td>
<td>23.6%</td>
</tr>
<tr>
<td>3.00- 3.99</td>
<td>283</td>
<td>28.7%</td>
</tr>
<tr>
<td>4.00- 4.99</td>
<td>265</td>
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</tr>
<tr>
<td>5.00- 5.99</td>
<td>299</td>
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</tr>
<tr>
<td>8.00- 8.99</td>
<td>187</td>
<td>49.5%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>9.00- 9.87 and greater</td>
<td>441</td>
<td>43.9%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>3946</td>
<td>27.0%</td>
</tr>
</tbody>
</table>
2017 San Antonio Breast Cancer Symposium

Publication Number: PD3-06

Title: Association of intrinsic subtypes with pathological complete response (pCR) in the KRISTINE neoadjuvant phase 3 clinical trial in HER2-positive early breast cancer (EBC)

Aleix Prat¹, Dennis Slamon², Sara A Hurvitz², Michael F Press³, Gail Lewis Phillips⁴, Vanessa Lopez Valverde⁵, Astrid Kiermaier⁵, Hans-Joachim Helms⁵, Miguel Martin⁶ and Sanne L de Haas⁵. ¹Hospital Clinic of Barcelona, Barcelona, Spain; ²David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA; ³Keck School of Medicine, USC/Norris Comprehensive Cancer Center, Los Angeles, CA; ⁴Genentech, Inc., South San Francisco, CA; ⁵F. Hoffmann-La Roche Ltd., Basel, Switzerland and ⁶Hospital Gregorio Marañón, Universidad Complutense, Madrid, Spain.

Body: Introduction: Previous studies have shown that HER2+ breast cancer is biologically heterogenous and intrinsic subtypes can be identified (luminal A, luminal B, HER2-enriched [HER2-E] and basal-like). HER2-E predominates and is associated with higher response rates following anti-HER2-based chemotherapy or dual HER2 blockade (only with lapatinib and trastuzumab). We explored the ability of intrinsic subtypes to predict pCR in pts treated with anti-HER2 neoadjuvant therapy.

Methods: KRISTINE (NCT02131064) is an open-label, phase 3 study of neoadjuvant trastuzumab emtansine + pertuzumab (T-DM1+P) vs docetaxel + carboplatin + trastuzumab + pertuzumab (TCH+P) in pts with HER2+ EBC. Treatment-naive pts with stage II–IIIc HER2+ EBC were randomized to receive 6 cycles of T-DM1+P or TCH+P and assessed for the primary endpoint, pCR (ypT0/is, ypN0). HER2 and hormone receptor (HR) status were centrally assessed. Gene expression (RNA) was assessed by a custom 800-gene codeset on the nCounter platform. Intrinsic subtypes were assessed with the research-based PAM50 classifier.

Results: KRISTINE randomized 444 pts (data cutoff, Dec 3, 2015; TCH+P, n=221; T-DM1+P, n=223). PAM50 results were available for 354 pts (79.7% of the intent-to-treat [ITT] population). Baseline characteristics and efficacy in the PAM50 population were similar to that of the ITT population. The HER2-E subtype represented 54.8% of the samples (Table 1). Differences were observed by HR status. Almost all luminal tumors (131/132) were identified within HR+ disease. Of HR+ tumors, 32% were identified as HER2-E. In the TCH+P arm, the pCR rate was 72.1% for HER2-E vs 32.8% in the other subtypes combined (Table 2.) In the T-DM1+P arm, the pCR rate was 62.2% for HER2-E vs 26.9% in the other subtypes combined. No major differences were observed in pCR rates within the HER2-E subtype according to HR status. Further multivariable analyses assessing differences between treatment arms and treatment benefit across subtypes is ongoing.

Table 1. Intrinsic subtypes

<table>
<thead>
<tr>
<th>Intrinsic subtypes</th>
<th>ITT (n=354)</th>
<th>HR- (n=143)*</th>
<th>HR+ (%) (n=200)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2-E</td>
<td>194 (54.8)</td>
<td>123 (86.0)</td>
<td>64 (32.0)</td>
</tr>
<tr>
<td>Luminal A</td>
<td>60 (16.9)</td>
<td>1 (0.7)</td>
<td>57 (28.5)</td>
</tr>
<tr>
<td>Luminal B</td>
<td>74 (20.9)</td>
<td>0</td>
<td>74 (37.0)</td>
</tr>
<tr>
<td>Basal-like</td>
<td>26 (7.3)</td>
<td>19 (13.3)</td>
<td>5 (2.5)</td>
</tr>
</tbody>
</table>

*Central HR status unknown for n=11

Table 2. pCR by intrinsic subtype

<table>
<thead>
<tr>
<th>Intrinsic subtypes</th>
<th>TCH+P</th>
<th>T-DM1+P</th>
<th>pCR difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>pCR, n (%)</td>
<td>n</td>
<td>pCR, n (%)</td>
</tr>
<tr>
<td>HER2-E</td>
<td>104</td>
<td>75 (72.1)</td>
<td>90</td>
</tr>
<tr>
<td>Subtype</td>
<td>HER2-E and HR+ *</td>
<td>HER2-E and HR- *</td>
<td>Other subtypes combined</td>
</tr>
<tr>
<td>---------------------------------------</td>
<td>------------------</td>
<td>------------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td><strong>Counts</strong></td>
<td>37</td>
<td>64</td>
<td>67</td>
</tr>
<tr>
<td><strong>Counts (as % of total)</strong></td>
<td>24 (64.9)</td>
<td>48 (75.0)</td>
<td>22 (32.8)</td>
</tr>
<tr>
<td><strong>HR Status</strong></td>
<td>27</td>
<td>59</td>
<td>93</td>
</tr>
<tr>
<td><strong>HR Status (as % of total)</strong></td>
<td>15 (55.6)</td>
<td>37 (62.7)</td>
<td>25 (26.9)</td>
</tr>
<tr>
<td><strong>pCR Rate</strong></td>
<td>-9.31 (-33.56, 14.94)</td>
<td>-12.29 (-28.56, 3.98)</td>
<td>-5.9 (-20.36, 8.45)</td>
</tr>
</tbody>
</table>

*Central HR status unknown for n=7

**Conclusions:** In this analysis from the KRISTINE study, HER2-E was the most common intrinsic subtype and was associated with the highest pCR rate with both regimens. Results are consistent with previous findings. The luminal A and B subtypes were well associated with HR+ status. A sizeable subgroup of the HER2-E subtype was HR+ (32%), and pCR rates within the HER2-E subtype seemed independent of HR status.
2017 San Antonio Breast Cancer Symposium

Publication Number: PD3-07

**Title:** Safety and efficacy results from a phase 1 study of DS-8201a in patients with HER2 expressing breast cancers

Shamu Modi¹, Junji Tsurutani², Shunji Takahashi³, Hiroji Iwata⁴, Haeseong Park⁵, Charles H Redfern⁶, Toshihiko Doi⁷, Bob Li¹, Tsutomu Iwasa², Shunichiro Taira³, Masaya Hattori⁴, Cynthia X Ma⁵, Jennifer M Fisher⁶, Yoichi Naito⁷, Kan Yonemori⁰, Yui Kawasaki⁰, Kaku Saito⁰, Takahiro Jikoh₁⁰, Javad Shahidi¹⁰, Caleb C Lee¹⁰, Antoine Yver¹⁰ and Kenji Tamura⁸. ¹Memorial Sloan-Kettering Cancer Center, New York, NY; ²Kindai University Hospital, Osakasayama, Osaka, Japan; ³The Cancer Institute Hospital of Japanese Foundation for Cancer Research, Kotoku, Tokyo, Japan; ⁴Aichi Cancer Center Hospital, Nagoya, Aichi, Japan; ⁵Washington University School of Medicine, St Louis, MO; ⁶Sharp Memorial Hospital, San Diego, CA; ⁷National Cancer Center Hospital East, Kashiwa, Chiba, Japan; ⁸National Cancer Center Hospital, Chuoku, Tokyo, Japan; ⁹Daiichi Sankyo Co Ltd, Shinagawaku, Tokyo, Japan and ¹⁰Daiichi Sankyo Inc, Basking Ridge, NJ.

**Body:**

**Background**

DS-8201a is a HER2 targeting antibody-drug conjugate (ADC) with high drug to antibody ratio (8:1), a novel linker and topoisomerase I inhibitor as the payload. In preclinical studies, DS-8201a showed efficacy against both trastuzumab emtansine (T-DM1) resistant HER2 positive breast cancer (BC) and against BC with low HER2 expression. The current phase 1 trial is comprised of dose escalation (Part 1) and dose expansion (Part 2) cohorts including patients (pts) with BC, gastric cancer and other HER2 expressing tumors. Only the results from BC cohorts are presented here.

**Methods**

Part 1 used a modified continual reassessment model to identify the recommended phase 2 dose (RP2D) of DS8201a in pts with BC or gastric cancer of any HER2 status. Two out of planned 4 expansion cohorts included BC: T-DM1-treated HER2 positive BC (Cohort 2a; IHC 3+ or IHC 2+/ISH +) and HER2 low BC (Cohort 2c, IHC 2+/ISH- or IHC 1+). Efficacy endpoints of objective response rate (ORR), disease control rate (DCR: CR + PR + SD), durability of response, progression free survival (PFS) and adverse events (AEs) were assessed.

**Results**

Overall, 146 subjects were included (24 Part 1; 122 Part 2). DS-8201a was administered up to 8.0 mg/kg in Part 1 without any DLTs, maximum tolerated dose was not reached. Based on available results, the dose levels of 5.4 and 6.4 mg/kg IV every 3 weeks were chosen for Part 2. In Part 2, a total of 56 BC pts were enrolled; 46 pts in Cohort 2a and 10 pts in Cohort 2c. The median age was 56 and number of prior regimens in the metastatic setting was 5 (range: 1-16). 37 BC pts had follow-up imaging scans and were evaluable for response at the time of data cutoff. Confirmed ORR was 41% (15/37) including 1 CR; DCR was 97% (36/37). The median duration of treatment was not reached. For Cohort 2a, where all pts had received prior T-DM1, treatment with DS-8201a achieved a higher ORR of 41% (11/27) and DCR of 100% (27/27) compared to the reported response these patients had to their prior T-DM1 treatment with ORR of 23% (5/22) and DCR of 73% (16/22). In the subset of 24 pts from cohort 2a who had received prior treatment with pertuzumab and T-DM1, the confirmed ORR was 44% (11/25). In Cohort 2c, confirmed ORR was 40% (4/10), DCR was 90% (9/10). Of the 46 BC pts in Part 2 who received at least one dose of DS-8201a, 4 pts discontinued treatment due to disease progression and 3 pts discontinued due to AE. The most common AEs of any grades were nausea, decreased appetite, vomiting, alopecia, and diarrhea. Only 2 pts experienced grade 4 AEs (thrombocytopenia and neutropenia) and 46 % (21/46) experienced grade 3 AEs (most commonly anemia, neutropenia, thrombocytopenia, leukopenia, lymphocytopenia, and vomiting).

**Conclusions**

DS-8201a was well tolerated and has significant activity in pts with T-DM1 and T-DM1/ pertuzumab pretreated HER2 positive BC and in pts with HER2 low BC, with durable disease control. Promising efficacy of this ADC in BC warrants further investigation.
Title: A randomized phase II trial of pyrotinib plus capecitabine versus lapatinib plus capecitabine in patients with HER2-positive metastatic breast cancer previously treated with taxanes, anthracyclines and/or trastuzumab

Body: Background: Pyrotinib is an oral, irreversible pan-ErbB receptor tyrosine kinase inhibitor (TKI) with activity against epidermal growth factor receptor (EGFR) / HER1, HER2, and HER4. Lapatinib in combination with capecitabine is one of the standards of care for patients with HER2-positive metastatic breast cancer (MBC) who have received prior taxanes, anthracyclines and/or trastuzumab.

Methods: We conducted an open label, multicenter, randomized phase II trial to comparatively evaluate efficacy and safety of pyrotinib + capecitabine (PC) or lapatinib + capecitabine (LC) in women with HER2-positive MBC. Key eligibility criteria included prior treatment with taxanes, anthracyclines and/or trastuzumab, ≤2 prior chemotherapies for metastatic disease, no CNS metastases, and no prior treatment with HER2 targeted TKI. Eligible patients were randomized 1:1 to PC Arm (P 400 mg QD D1–21 + C 1000 mg/m² BID D1–14, 21-D cycle) or LC Arm (L 1250 mg QD D1–21 + C 1000 mg/m² BID D1–14, 21-D cycle). The primary endpoint was objective response rate (ORR) as assessed by investigator, and secondary endpoints included progression-free survival (PFS), time to progression (TTP), duration of response (DoR), overall survival (OS), and safety.

Results: Between May 2015 and Mar 2016, 128 patients (65 in PC arm and 63 in LC arm) were enrolled in this study. Median age was 48 years (range 25-70), ECOG performance status was 0 (53.9%) or 1 (46.1%), 62.5% had hormone receptor-positive disease, 76.6% had visceral disease and 53.9% had received prior trastuzumab in (neo)adjuvant and/or metastatic setting. Baseline characteristics were well balanced in two arms. Median follow-up time was 15.0 months. ORR was 78.5% in PC arm and 57.1% in LC arm (p=0.01), Median PFS was 18.1 months in PC arm and 7.0 months in LC arm (hazard ratio 0.363; 95% CI 0.228, 0.579; p<0.0001), PFS benefits in PC arm compared to LC arm was observed irrespective of prior trastuzumab or not. Treatment related Grade 3-4 toxicities occurred in >2% patients in PC arm vs LC arm included hand-foot syndrome (21.5% vs 19.0%), diarrhea (13.8% vs 4.8%), decreased neutrophil (7.7% vs 1.6%), decreased WBC (6.2% vs 1.6%), vomiting (4.6% vs 0%), increased AST (3.1% vs 1.6%), decreased hemoglobin (3.1% vs 1.6%), increased total bilirubin (0% vs 4.8%) and increased conjugated bilirubin (0% vs 3.2%).

Conclusions: In women with HER2-positive MBC previously treated with taxanes, anthracyclines and/or trastuzumab, pyrotinib + capecitabine yield statistically significant better PFS and ORR than lapatinib + capecitabine in this randomized phase II trial. Phase III study is ongoing to validate this finding.
Title: Pertuzumab and trastuzumab with or without metronomic chemotherapy for older patients with HER2-positive metastatic breast cancer: Results from the EORTC 75111-10114 ETF/BCG randomized phase II study

Hans Wildiers¹, Konstantinos Tryfonidis², Lissandra dal Lago³, Peter Vuylsteke⁴, Giuseppe Curigliano⁵, Simon Waters⁶, Barbara Brouwers⁷, Kim Aalders², Bart Meulemans², Saskia Litiere², Nathan Touati⁶, Fatima Cardoso⁸ and Etienne Brain⁹.

¹University Hospitals Leuven and KU Leuven, Leuven, Belgium; ²European Organization for Research and Treatment of Cancer (EORTC Headquarters), Brussels, Belgium; ³Institute Jules Bordet, Brussels, Belgium; ⁴Centre Hospitalier Universitaire, Universite Catholique de Louvain, Namur, Belgium; ⁵Istituto Europeo di Oncologia, Milan, Italy; ⁶Velindre NHS Trust- Velindre Cancer Centre, United Kingdom; ⁷AZ Sint- Jan Hospital, Brugge, Belgium; ⁸Champalimaud Clinical Center, Champalimaud Foundation, Lisbon, Portugal and ⁹Institut Curie- Hopital Rene Huguenin, Saint Cloud, Paris, France.

Body: Introduction: Pertuzumab (P) is approved as first line therapy for HER2-positive (HER2+) metastatic breast cancer (MBC) combined with trastuzumab (T) and docetaxel. However older patients are at higher risk of chemotherapy-induced toxicity raising high interest in a less toxic backbone such as metronomic chemotherapy and in chemo-free dual HER2 blockade (TP). Patients and Methods: This phase II selection study randomized (1:1) patients with HER2+ MBC, aged 70+ or frail 60+, to first line chemotherapy with metronomic oral cyclophosphamide 50 mg/day + TP (TPM) or TP alone. Prior endocrine therapy and up to 1 line of anti-HER2 therapy (without chemotherapy) for MBC were allowed. T-DM1 was offered in case of progression. Randomization was stratified according to hormonal receptors, previous anti-HER2 treatment and geriatric assessment. Primary endpoint was progression-free survival (PFS) rate at 6 months seeking a difference of ≥10% between the two arms. Results: Between July 2013 and May 2016, 39 and 41 patients were randomized to TP and TPM arm respectively: median age 76.7 years, hormone receptor positivity 69%, prior adjuvant T 11%, prior metastatic T (with endocrine therapy) 3%, visceral involvement 93.7%, potential frailty profile according to geriatric screening G8 (≤14) 71% and/or to short physical performance battery (<10) 81%, Charlson comorbidity score > 0 in 40%. With 20.7 months of median follow-up, 6-month and median PFS were 46.2% (95% CI 30.2-60.7) and 5.6 months (95% CI 3.6-16.8) versus 73.4% (95% CI 56.6-84.6) and 12.7 months (95% CI 6.7-24.8) for TP and TPM, respectively. Four patients in TPM and 2 in TP developed brain metastases only as progression event. OS and breast cancer specific survival were comparable between the two arms; 9/29 deaths were not breast cancer-related. Response rate was 44% in TP arm and 53% in TPM arm. In 29 patients who received T-DM1 second line, 6-month PFS, median PFS and response rate were 49.5% (95% CI 29.2-66.9), 5 months (95% CI 2.5-12.5) and 13.5%. In patients who discontinued TP(M), 37, 9 and 14 stopped because of progression, toxicity or other reasons, respectively. During TPM treatment, 1 patient died of heart failure and 1 developed grade 3 heart failure; 1 patient in each arm developed a ≥10% asymptomatic left ventricular ejection fraction decrease below 50%. Diarrhea any grade and grade ≥3 were observed in 56% and 8% versus 71% and 12% patients in TP and TPM arms, respectively. No grade 3 or febrile neutropenia was reported. There was no relevant difference in functional evolution between both groups. In the whole population, several geriatric items were of prognostic value by multivariate analysis: e.g. for OS, G8 >14 vs ≤ 14 HR=0.12 (95% CI 0.03-0.55, p 0.006). In 29 patients receiving T-DM1, grade 3 toxicity was rare: fatigue (2 patients), thrombocytopenia and epistaxis (1 patient). Conclusions: Metronomic chemotherapy-based dual blockade (TPM) seems to be superior to dual blockade alone (TP) in an elderly/frail HER2+ MBC population, with an attractive safety profile. TPM, followed by T-DM1 after progression, may delay or supersede taxane chemotherapy in this population.
2017 San Antonio Breast Cancer Symposium

Publication Number: PD3-10

Title: Safety results from a randomized, double-blind, phase 3 study of ABP 980 compared with trastuzumab in patients with breast cancer

Hans-Christian Kolberg¹, Georgia S Demetriou², Nan Zhang³, Zorica Tomasevic⁴ and Vladimir Hanes³. ¹Marienhospital gGmbH, Klinik fur Gynakologie und Geburtshilfe, Bottrop, Germany; ²Charlotte Mexeke Johannesburg Academic Hospital Ward, Johannesburg Gauteng, South Africa; ³Amgen, Inc., Thousand Oaks, CA and ⁴Institute for Oncology and Radiology of Serbia, Belgrade, Serbia.

Body: Background: Analytical, functional, and pharmacokinetic similarity between the proposed biosimilar ABP 980 and trastuzumab (TRAS) has been shown. Similar efficacy and safety have also been demonstrated in the neoadjuvant phase of the phase 3 comparative clinical study. Here we focus on the safety and immunogenicity results from the adjuvant phase of this study.

Methods: The objective of this study was to compare ABP 980 with TRAS in women with HER2 positive early breast cancer. After run-in anthracycline-based chemotherapy, patients were randomized 1:1 to intravenous ABP 980 or TRAS plus paclitaxel Q3W for 4 cycles. Surgery was completed 3-7 weeks after the last dose of neoadjuvant study drug. After surgery, patients who initially received TRAS were allocated to either continue TRAS (TRAS/TRAS arm) or undergo a single switch to receive ABP 980 (TRAS/980 arm) and patient who initially received ABP 980 continued to receive ABP 980 (980/980 arm) Q3W for up to 1 year. This report is based on safety data collected at the time of primary analysis, when all patients had completed the first post-surgery clinical visit or had withdrawn. The majority of patients had completed the study at the time of this analysis.

Results: 725 patients were randomized; 364 and 361 patients were randomized to ABP 980 and TRAS, respectively, in the neoadjuvant phase. Following surgery, 349 patients in the 980/980 arm, 171 patients in the TRAS/TRAS arm, and 171 patients in the TRAS/980 arm entered the adjuvant phase. Adverse events (AEs) during the adjuvant phase are shown in Table 1; data from the neoadjuvant safety phase have been previously reported.

Table 1

<table>
<thead>
<tr>
<th>AE Category</th>
<th>980/980 (N = 349)</th>
<th>TRAS/TRAS (N = 171)</th>
<th>TRAS/980 (N = 171)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any AE</td>
<td>201 (57.6)</td>
<td>89 (52.0)</td>
<td>98 (57.3)</td>
</tr>
<tr>
<td>Any grade ≥3 AE</td>
<td>27 (7.7)</td>
<td>10 (5.8)</td>
<td>10 (5.8)</td>
</tr>
<tr>
<td>Any fatal AE</td>
<td>0</td>
<td>0</td>
<td>1 (0.6)</td>
</tr>
<tr>
<td>Any serious AE</td>
<td>14 (4.0)</td>
<td>4 (2.3)</td>
<td>4 (2.3)</td>
</tr>
<tr>
<td>AEs of Interest, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infections and infestations</td>
<td>46 (13.2)</td>
<td>14 (8.2)</td>
<td>21 (12.3)</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>37 (10.6)</td>
<td>16 (9.4)</td>
<td>13 (7.6)</td>
</tr>
<tr>
<td>Infusion reactions</td>
<td>27 (7.7)</td>
<td>10 (5.8)</td>
<td>15 (8.8)</td>
</tr>
<tr>
<td>Hypersensitivity</td>
<td>11 (3.2)</td>
<td>3 (1.8)</td>
<td>7 (4.1)</td>
</tr>
<tr>
<td>Pulmonary toxicity</td>
<td>3 (0.9)</td>
<td>2 (1.2)</td>
<td>1 (0.6)</td>
</tr>
<tr>
<td>Cardiac failure</td>
<td>0</td>
<td>1 (0.6)</td>
<td>1 (0.6)</td>
</tr>
<tr>
<td>LVEF decline by ≥10% and to below 50%, n/N* (%)</td>
<td>9/313 (2.9)</td>
<td>3/154 (1.9)</td>
<td>3/153 (2.0)</td>
</tr>
</tbody>
</table>

LVEF = left ventricular ejection fraction; N* = Number of patients with data available

One patient in the TRAS/980 arm developed binding antibodies during the adjuvant phase; this patient tested negative for neutralizing antibodies. In the neoadjuvant phase, 2 patients in the ABP 980 arm and 2 in the TRAS arm developed binding
antibodies but tested negative for neutralizing antibodies. The percent of patients with disease progression or recurrence or death was 5.2% in the 980/980 arm, 5.3% in the TRAS/TRAS arm, and 2.9% in the TRAS/980 arm. The estimated hazard ratio from a stratified Cox proportional hazards regression model for 980/980 versus TRAS/TRAS was 1.01 (90% CI: [0.530, 1.930]) and for TRAS/980 versus TRAS/TRAS was 0.48 (90% CI: [0.181, 1.292]).

**Conclusions:** Safety of ABP 980 is similar to TRAS in patients with HER2+ early breast cancer in both the neoadjuvant and adjuvant phases and consistent with the historical safety profile of TRAS. The immunogenicity profile of ABP 980 is low and consistent with TRAS.
Title: Efficacy and safety of subcutaneous or intravenous trastuzumab in patients with HER2-positive early breast cancer after 5 years' treatment-free follow-up: Final analysis from the phase III, open-label, randomized HannaH study

Christian Jackisch¹, Daniil Stroyakovskiy², Xavier Pivot³, Jin-Seok Ahn⁴, Bohuslav Melichar⁵, Shin-Cheh Chen⁶, Christoph Meyenberg⁷, Nedal Al-Sakaff⁸, Dominik Heinzmann⁸ and Roberto Hegg⁹. ¹Sana Klinikum Offenbach GmbH, Breast Cancer and Gynecology Cancer Center, Offenbach, Germany; ²City Clinical Oncology Hospital 62, Moscow, Russian Federation; ³CHU Jean Minjoz, Chemotherapy – Oncology, Besançon, France; ⁴Samsung Medical Center and Sungkyunkwan University School of Medicine, Seoul, Korea; ⁵Palacky University Medical School & Teaching Hospital, Olomouc, Czech Republic; ⁶Chang Gung Memorial Hospital, Taoyuan, Taiwan; ⁷F. Hoffmann-La Roche Ltd, Basel, Switzerland; ⁸Product Development – Oncology, F. Hoffmann-La Roche Ltd, Basel, Switzerland and ⁹Hospital Pérola Byington, São Paulo, Brazil.

Body: Background HannaH (NCT00950300) compared subcutaneous and intravenous trastuzumab (H SC and H IV) as neoadjuvant–adjuvant therapy for HER2-positive breast cancer. The co-primary endpoints of pathological complete response (pCR) and serum trough concentration at predose cycle 8 demonstrated noninferiority between H SC and H IV. Efficacy analyses of event-free survival (EFS) and overall survival (OS) at a median follow-up of 40 months supported this noninferiority. Safety analyses also confirmed the consistency of the safety profile across both arms. In this final follow-up analysis, we report the long-term efficacy and safety outcomes at 5 years of treatment-free follow-up (TFFU; 6 years in total). The correlation between total pCR (tpCR; absence of invasive neoplastic cells in ipsilateral nodes and the breast) and EFS was also explored.

Methods Enrolled patients (n=596; pts) were randomized to receive 4 cycles of docetaxel, then 4 cycles of 5-fluorouracil/epirubicin/cyclophosphamide concurrently with 3-weekly fixed-dose 600mg H SC or H IV (loading: 8mg/kg; maintenance: 6mg/kg) in the neoadjuvant setting. Post-surgery, pts received an additional 10 cycles of H SC or H IV in the adjuvant setting to complete 1 year of anti-HER2 therapy. EFS (time from randomization to local, regional, or distant recurrence, contralateral breast cancer, or death) and OS were calculated using the Kaplan-Meier method. Adverse events (AEs) and serious AEs were recorded and graded per standard criteria.

Results In total, 297 pts were randomized to the H SC arm and 299 to the H IV arm; 294 and 297 pts were included in the respective efficacy analysis populations. Median duration of follow-up (including TFFU) was 70.8 and 71.4 months in the H SC and H IV arms, respectively. EFS and OS were similar across both study arms (Table 1). Pts who achieved tpCR had longer EFS and OS vs. those who did not (Table 1).

| Table 1 |
|-----------------|-----------------|-----------------|
| 6-year EFS, % (95% CI) | H SC | H IV | Hazard Ratio (95% CI) |
| Overall | n=294 | n=297 | 0.98 (0.74;1.29) |
| tpCR status* | tpCR | n=102 | n=90 |
| | 80 (73;88) | 83 (75;91) |
| | no tpCR | n=158 | n=173 |
| | 57 (49;65) | 61 (54;69) |
| 6-year OS, % (95% CI) | H SC | H IV | 0.94 (0.61;1.45) |
| Overall | n=294 | n=297 |
| * Efficacy per protocol population |

Cardiac AE incidence was low and consistent across study arms (Table 2).

Table 2
<table>
<thead>
<tr>
<th>Pts, n (%)</th>
<th>H SC (n=297)</th>
<th>H IV (n=298)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any AE</td>
<td>290 (98)</td>
<td>282 (95)</td>
</tr>
<tr>
<td>≥ Grade 3 AE</td>
<td>158 (53)</td>
<td>160 (54)</td>
</tr>
<tr>
<td>Serious AE</td>
<td>65 (22)</td>
<td>45 (15)</td>
</tr>
<tr>
<td>Cardiac AE</td>
<td>44 (15)</td>
<td>42 (14)</td>
</tr>
<tr>
<td>LVEF decline (≥10%-points from baseline to &lt;50%)</td>
<td>11 (3.8)</td>
<td>12 (4.2)</td>
</tr>
</tbody>
</table>

LVEF, left ventricular ejection fraction

**Conclusion** Long-term efficacy EFS and OS results confirmed the noninferiority of H SC compared with H IV, as demonstrated by pCR and pharmacokinetic endpoints. tpCR was associated with longer EFS and OS. The overall safety profile of H SC was consistent with that of H IV.
Title: PIK3CA alterations and benefit with neratinib after trastuzumab-based adjuvant therapy in early-stage HER2+ breast cancer: Correlative analyses of the phase III ExteNET trial


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Body: Background: Neratinib is a pan-HER tyrosine kinase inhibitor that blocks the PI3K/Akt and MAPK signaling pathways downstream from HER2. The international, randomized, placebo-controlled phase III ExteNET trial showed that a 1-year course of neratinib after trastuzumab-based adjuvant therapy significantly improved 2-year invasive disease-free survival (iDFS) in early-stage HER2+ breast cancer (HR 0.67; 95% CI 0.50–0.91; p=0.0091) [Chan et al. Lancet Oncol 2016]. Furthermore, the effects of neratinib on iDFS were shown to be durable at 5 years' follow-up (HR 0.73; 95% CI 0.57–0.92; p=0.008) [Martin et al. ESMO 2017]. PIK3CA alterations are common in HER2+ breast cancers, and in general are associated with a worse prognosis. We sought to assess the prognostic and predictive significance of PIK3CA alterations in an exploratory substudy of the ExteNET trial.

Methods: ExteNET is an international, multi-center, randomized, double-blind, placebo-controlled phase III trial (Clinicaltrials.gov: NCT00878709). Patients received oral neratinib 240 mg/day or placebo for 1 year. Of the intent-to-treat (ITT) population (n=2840), primary formalin-fixed paraffin-embedded (FFPE) tumor specimens were available from 991 patients for PIK3CA mutation testing by RT-PCR for two hot-spot mutations in exon 9 (E542K, E545K/D) and one hot-spot mutation in exon 20 (H1047R). 702 FFPE tumor slides underwent FISH analysis for PIK3CA amplification with a ratio of ≥2.2 considered as amplified. Primary endpoint: iDFS. iDFS events were tested by 2-sided log-rank tests, and HR (95% CI) were estimated using Cox proportional-hazards models. Data cut-off: March 2017.

Results: Baseline demographics and disease characteristics between treatment arms of the correlative cohort (n=1201) were balanced. Overall, 21.2% (n=210) of primary tumors harbored one of the specified PIK3CA mutations, and 8.7% (n=61) were PIK3CA FISH-amplified. Patients with PIK3CA-altered tumors (i.e. PIK3CA mutations or FISH-amplified) had fewer iDFS events with neratinib compared with placebo (HR 0.41; 95% CI 0.17-0.90, p=0.028). The interaction test was not significant (p=0.1842). Results of the various correlative analyses within treatment arms are shown in the table.

<table>
<thead>
<tr>
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<th>Neratinib</th>
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<tr>
<td>PIK3CA-altered</td>
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Conclusions: One year of neratinib treatment after trastuzumab-based adjuvant therapy significantly improves iDFS after 5 years in patients with early-stage HER2+ breast cancer. From this modest-sized exploratory cohort, it appears that PIK3CA may be a biomarker for differential sensitivity to neratinib after 1 year of trastuzumab in the adjuvant setting. These exploratory results should be validated in a larger subset.
Title: Phase 1 study of CB-839, a first-in-class oral inhibitor of glutaminase, in combination with paclitaxel in patients with advanced triple negative breast cancer

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Body: Background: CB-839 is a first-in-class oral highly selective inhibitor of glutaminase (GLS), a mitochondrial enzyme that plays a key role in cancer cell metabolism. Triple negative breast cancer (TNBC) has high GLS expression and demonstrates high glutamine utilization and glutamine dependence in clinical and preclinical studies. CB-839 has monotherapy antitumor activity in vitro and in vivo in preclinical models of TNBC, and also demonstrates synergistic anti-cancer activity with the standard of care agent paclitaxel (Pac).

In an ongoing Phase 1 study, CB-839 combined with Pac (Pac-CB) is being evaluated in patients (pts) with metastatic TNBC. We previously reported that Pac-CB was well-tolerated and active in heavily pre-treated TNBC pts, including those previously refractory to taxane treatment for metastatic disease. We report here updated results from this ongoing study.

Methods: Pts with refractory metastatic TNBC (prior taxane therapy allowed) received escalating doses of CB-839 (400-800 mg BID) in combination with full dose Pac of 80 mg/m² Days 1, 8, 15 every 28 days. After demonstration of safety and tolerability, an expansion cohort was opened at the CB-839 recommended phase 2 dose of 800 mg PO BID.

Results: As of the May 2017 data cut, 45 pts have enrolled across the dose escalation and expansion cohorts (7 pts at 400 mg, 12 at 600 mg, and 26 at 800 mg). 15 pts (33%) have received ≥5 prior lines of systemic therapy for metastatic disease (median 3 prior lines), and 39 pts (87%) have received prior taxane therapy. The Pac-CB regimen has been well tolerated with the most frequent treatment-related Grade ≥3 AEs being neutropenia (24%), anemia (7.6%), fatigue (2.6%), WBC decreased (2.6%), and peripheral neuropathy (2.6%). At CB-839 doses ≥800 mg BID (n=27 RECIST-evaluable), the ORR has been 22% and disease control rate (DCR=CR+PR+SD) 59%; in the subgroup of pts refractory to previous taxane therapy (n=19) the ORR has been 21% (4 pts) and DCR 47% (9 pts). The highest ORR in this study has been seen in pts of African ancestry (AA, n=11 RECIST evaluable) with an ORR of 36% (4 pts) and DCR 55% (6 pts), with all 4 AA responders being refractory to prior taxane treatment for advanced/metastatic disease.

Conclusions: TNBC has elevated glutamine metabolism that requires mitochondrial GLS. In TNBC pts with heavy pretreatment and previous taxane exposure, the combination of CB-839 with Pac has demonstrated clinical activity including a 21% ORR in pts refractory to taxane. Notably, in patients of African ancestry, a population reported to have especially high glutamine utilization in TNBC tumors, the encouraging ORR of 36% suggests a potential genetic association with treatment response. The Pac-CB regimen has been well tolerated in late line TNBC pts, including a Gr 3 neuropathy signal of only 2.6%. A Phase 2 study, CX-839-007, has been initiated to further evaluate the activity and safety of Pac-CB in pts with TNBC, including defined cohorts of pts with 1st line and 3rd line+ metastatic disease in pts of African and non-African ancestry. Responses in relation to genetic background, molecular subtype of TNBC and glutamine biology will be studied.
Body: Background

LIV-1, a transmembrane protein and downstream target of STAT3, is highly expressed in breast cancer cells. It is associated with lymph node involvement and metastatic progression. SGN-LIV1A is an anti-LIV-1 antibody conjugated via a protease-cleavable linker to monomethyl auristatin E (MMAE). Upon binding to cell-surface LIV-1, SGN-LIV1A is internalized and releases MMAE, which disrupts microtubulin and induces apoptosis.

Methods

This ongoing, phase 1 study evaluates safety, tolerability, pharmacokinetics, and antitumor activity of SGN-LIV1A (q3wks IV) in women with LIV-1-positive, unresectable, locally advanced or metastatic breast cancer (LA/MBC) (NCT01969643). Patients (pts) with measurable disease and ≥2 prior cytotoxic regimens for LA/MBC are eligible. Pts with ≥ Grade 2 neuropathy are excluded. Response is assessed per RECIST v1.1; pts with stable disease (SD) or better can continue treatment until disease progression or intolerable toxicity. At completion of dose escalation in hormone receptor-positive/HER2-negative (HR+/HER2−) and triple-negative (TN) pts, expansion cohorts were opened to further evaluate safety and antitumor activity of monotherapy in TN pts. Tumor biopsies are evaluated for LIV-1 expression.

Results

To date, 69 pts (18 HR+/HER2−, 51 TN) have received a median of 3 cycles (range, 1–12) of SGN-LIV1A at doses of 0.5–2.8 mg/kg. Median age was 56 yrs. Pts had a median of 3 prior cytotoxic regimens for LA/MBC; 58 had visceral disease and 37 had bone metastases. No dose-limiting toxicities (DLTs) occurred in 19 DLT-evaluable pts; maximum tolerated dose was not exceeded at 2.8 mg/kg. Expansion cohorts of TN pts were opened at 2.0 and 2.5 mg/kg. Treatment-emergent adverse events (AEs) reported in ≥25% of pts were fatigue (59%), nausea (51%), peripheral neuropathy (44%), alopecia (36%), decreased appetite (33%), constipation (30%), abdominal pain, diarrhea, and neutropenia (25% each). Most AEs were Grade 1/2; AEs ≥ Grade 3 included neutropenia (25%) and anemia (15%). Febrile neutropenia occurred in 2 pts whose total dose exceeded 200 mg per cycle, including 1 treatment-related death due to sepsis. No other treatment-related deaths occurred on-study. Seven pts discontinued treatment due to AEs. In dose escalation, activity was observed in 17 efficacy evaluable (EE) HR+/HER2- pts, with a disease control rate (DCR= CR+PR+SD) of 59% (10 SD), including 1 pt with SD ≥24 wks. Among the 44 EE TN pts (dose escalation plus expansion cohorts), the objective response rate (ORR) was 32% (14 PR) with a confirmed PR rate of 21%, DCR was 64% (14 PR, 14 SD), and clinical benefit rate (CBR=CR+PR+SD ≥24 wks) was 36% (16 pts). For TN pts, median PFS was 11.3 wks (95% CI: 6.1, 17.1); 10 pts remain on treatment.

Of 631 MBC tumor samples of all clinical subtypes evaluated for LIV-1, 91% were positive; 75% had moderate-to-high expression (H-score ≥100).

Conclusions

LIV-1 is expressed in almost all MBC tumors. SGN-LIV1A monotherapy was generally well tolerated and showed encouraging antitumor activity in heavily pretreated TN MBC, with a PR rate of 32%, confirmed PR rate of 21%, and CBR (≥24 wks) of 36%.
Response duration data continue to evolve. Enrollment continues in the TN monotherapy expansion cohort.
Title: Novel cFlip inhibitor suppresses chemotherapy-induced breast cancer stem cell activity through blocking HIF1-α

Tim Robinson¹, Dan Turnham¹, Aleks Gruca¹, Luke Piggott¹ and Richard Clarkson¹.¹Cardiff University, United Kingdom.

Body: Background: The emergence of the cancer stem cell (CSC) hypothesis has helped to explain previously poorly understood clinical concepts such as metastases, late tumour recurrence and resistance to chemotherapy. Triple Negative Breast Cancer (TNBC) has the worst prognosis of all types of breast cancer with a more frequent relapse rate and reduced length of survival in metastatic disease. It has been shown to contain a higher proportion of CSCs than other types of breast cancer. Paclitaxel, a taxane in widespread use in breast cancer, induces apoptosis in a ligand-independent manner through the extrinsic apoptosis pathway. cFLIP is both an antagonist of this apoptosis pathway and has the ability to form aggregates that interfere with the ubiquitylation and subsequent degradation of both HIF1α and β-catenin- two molecules involved in CSC-signalling. Using a novel compound targeted against cFLIP, we wanted to assess whether its combination with paclitaxel effectively targeted CSCs, particularly in TNBC.

Methods: In vitro experiments were used to assess the effect on cell viability, mammosphere formation and ALDH+ of both chemotherapy and our novel agent, OH14. We then used siRNA-mediated knockdown of cFLIP to confirm an on-target effect. Annexin V assays were used to assess apoptosis and both Western blotting and qPCR was used to examine the effect of paclitaxel and OH14 on protein levels and gene expression of CSC-signalling pathways. Athymic mice were used to in serial dilution experiments and for in vivo treatment with paclitaxel and OH14 on a TNBC cell line.

Results: We established an in vitro model demonstrating that a wide range of chemotherapeutic agents (FEC, Paclitaxel and Docetaxel) increased CSC-like behaviour and the ALDH+ population in a broad range of breast cancer cell lines. A mathematical model demonstrated that chemotherapy increased the absolute number of CSCs after treatment suggesting that CSC-like signaling was being induced. OH14, our novel compound, sensitised Triple Negative Breast Cancer (TNBC) cell lines to paclitaxel by increasing its apoptotic effect but had a more profound effect on mammosphere formation and ALDH positivity. This suggests that it preferentially targets CSCs. Serial dilution experiments demonstrated that chemotherapy increased the tumour forming potential of a TNBC cell line in vivo and that this effect was abrogated with OH14. Treatment of the same cell line in vivo demonstrated that paclitaxel +/- OH14 successfully targeted tumours but that the paclitaxel tumours recurred whereas those subject to combined treatment remained undetectable. Further experiments examining CSC signalling showed that HIF1α-mediated signalling was increased by paclitaxel and abrogated by the addition of OH14.

Conclusion: cFLIP has a dual effect in both increasing apoptosis and targeting signaling in TNBC CSCs. In a breast cancer subtype in desperate need of novel therapeutic strategies, targeting cFLIP warrants further investigation and progression towards clinical trials.
Clinical safety and efficacy of the aurora and angiogenic kinase inhibitor ENMD-2076 in previously treated, locally advanced or metastatic triple-negative breast cancer

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Body: Background: Triple-negative breast cancer (TNBC) is an aggressive breast cancer subtype defined by the lack of expression of the estrogen and progesterone receptors and lack of HER2 over-expression. ENMD-2076 is an orally bioavailable small molecule inhibitor of Aurora and angiogenic kinases with pro-apoptotic and antiproliferative activity in preclinical models of TNBC.

Methods: This two institution, single-arm, two-stage, phase II clinical trial enrolled patients with locally advanced or metastatic TNBC refractory to 1-3 prior lines of chemotherapy in the advanced setting. Patients had ECOG PS $\leq$ 1, measureable disease by RECIST 1.1 and no evidence of brain metastasis. Patients were treated with ENMD-2076 250 mg PO daily with continuous dosing in 4-week cycles until disease progression or unacceptable toxicity occurred. The primary end point was 6-month clinical benefit rate (6-CBR) and secondary endpoints included time to progression (TTP), PK profile, safety and biologic correlatives in archival and fresh serial tumor biopsies in a subset of patients.

Results: Between July 2012 and October 2016, 41 patients were enrolled (median age 54; range 30-73; female 40; male 1). Patients received a mean 1.7 prior lines of chemotherapy for locally advanced unresectable or metastatic disease and 80.5% received prior neoadjuvant or adjuvant chemotherapy (N=33). Thirty-six patients were evaluable per protocol for the primary efficacy analysis. Five patients (12.2%) were not included in the efficacy analysis due to: adverse events (AE) leading to discontinuation prior to objective efficacy assessment (N=3), not meeting eligibility criteria on day 1 (N=1) and withdraw of consent in cycle 1 (N=1). The study proceeded to the second stage of enrollment based on observing three 6-CBR events in Stage 1 (N=18 patients). The 6-CBR in the overall trial was 16.7% (95% exact CI: 6%-32.8%; 2 patients with PR and 4 patients with SD > 6 mos). The median duration of response or clinical benefit in these patients was 32 weeks (8 cycles). 4-CBR was 27.8% (95% exact CI: 14%-45.2%). Dose reduction occurred in 8 patients (20%) for fatigue, hypertension and proteinuria. The most common grade 3 treatment-related adverse events were hypertension (37.5%) and fatigue (10%). One patient experienced grade 4 hypertension. Analysis of serial tumor biopsies prior to and following 2 weeks of ENMD-2076 (N=8 patients), demonstrated a treatment-induced decrease in cellular proliferation (Ki-67) and microvessel density (CD34) as assessed by IHC. Immunofluorescence performed on a subset of samples demonstrated an increase in p53-family member expression following treatment, consistent with changes observed in preclinical TNBC patient-derived tumor xenograft models.

Conclusions: ENMD-2076 has durable clinical activity in a subset of patients with pretreated, advanced or metastatic triple-negative breast cancer. Predictive biomarker development using archival and fresh tumor tissue is underway. Exploration of lower doses of ENMD-2076 in future clinical trials may improve tolerability.
Title: The NSABP/NRG 8-gene signature accurately predicts degree of benefit from trastuzumab in Alliance/NCCTG N9831: Validation of the 8-gene signature in an independent clinical trial

Katherine L Pogue-Geile, Nan Song, Daniel J Serie and E Aubrey Thompson. 1NSABP/NRG Oncology, Pittsburgh and 2Mayo Clinic Comprehensive Cancer Center, Jacksonville.

Body: BACKGROUND
Clinical trials NRG/NSABP B-31 and Alliance/NCCTG N9831 demonstrated that trastuzumab added to chemotherapy extended disease-free survival (DFS) in breast cancer patients (pts) in the adjuvant setting (Romond et al). However, the degree of trastuzumab benefit varies among pts. We previously described an 8-gene model that was predictive for trastuzumab benefit, which was validated in an independent cohort of B-31 patients (Pogue-Geile 2013). The 8-gene signature subtyped B-31 pts in an independent validation cohort into three trastuzumab benefit groups: one with large benefit HR=0.27, one with medium benefit HR=0.56, and one with little to no benefit HR=1.56. The purpose of this study was to validate the 8-gene model in pts enrolled into N9831, which tested the efficacy of adding trastuzumab to doxorubicin plus cyclophosphamide → paclitaxel by randomizing pts into one of three arms: chemotherapy only (Arm A), trastuzumab given after chemotherapy (Arm B), or trastuzumab given concurrently with chemotherapy (Arm C).

METHODS
NCounter data, consisting of 8 predictive genes and 4 house-keeping genes, for 1,379 patients enrolled into N9831 were used to assign each patient to one of the three benefit groups. Assignments to one of the three trastuzumab benefit groups based on the 8-gene model were made by the NSABP/NRG Pathology Laboratory Biostatistician, who was blinded to outcome data. Predictions were sent to N9831 investigators and accuracy of predictions were tested in pts enrolled into arms A and C (N=892) using Cox models adjusted for age, nodes, ER/PR status, tumor size, and grade. Recurrence-free survival (RFS) and DFS were used as endpoints.

RESULTS
Tumors from N9831 were placed into one of the three trastuzumab benefit groups based on the 8-gene signature. The N9831 pts in the predicted-large benefit group had a hazard ratio (HR) of 0.47, P=0.0006, pts in the predicted-medium benefit group had an HR of 0.6, P=0.02, and the predicted-low benefit group had an HR of 1.54, P=0.375. The interaction P value was significant at 0.019 in adjusted Cox models. The RFS at 10 years for trastuzumab-treated pts was 72%, 83%, and 83% in the low, medium, and large benefit groups, respectively.

CONCLUSIONS
The 8-gene signature developed in a discovery cohort and validated in an independent cohort of B-31 pts has now been validated in N9831. Many anti-HER2 therapies such as lapatinib, afatinib, neratinib, pertuzumab, and TDM-1 have been approved for treatment in metastatic breast cancer pts and although these agents have shown responses, the actual improvements in outcomes in pts is variable and it is not known which pts actually receive benefit. Thus this signature may be clinically useful in identifying pts who may benefit from additional treatment beyond trastuzumab or for stratification of pts enrolled into clinical trials testing new anti-HER2 therapies.

SUPPORT
U10CA180868, -180822, UG1CA189867, U24CA196067, the PA DOH, which disclaims certain responsibilities, Genentech, Inc., and the Breast Cancer Research Foundation.
Title: The role of FGF/FGFR axis in resistance to SERDs and CDK4/6 inhibitors in ER+ breast cancer

Pingping Mao\textsuperscript{1,2}, Justin Kusiel\textsuperscript{1}, Ofir Cohen\textsuperscript{1,2} and Nikhil Wagle\textsuperscript{1,2}. \textsuperscript{1}Dana Farber Cancer Institute and \textsuperscript{2}Broad Institute.

Body: Approximately 70\% of breast cancers express the estrogen receptor (ER), and estrogen signaling drives breast cancer cell growth and progression. ER-directed therapies are commonly used to treat ER+ breast cancer and have improved survival for patients, yet resistance to those therapies inevitably occurs. Mutations in the estrogen receptor itself occur in \textasciitilde25-30\% of patients with ER+ metastatic breast cancer that has developed resistance to aromatase inhibitors. Beyond these ER mutations, other resistance mechanisms are not well described. Moreover, clinical mechanisms of resistance to another class of ER-targeted agents, selective estrogen receptor degraders (SERDs), such as fulvestrant have not been clearly identified.

Here we report two FGFR2 mutations identified in patients with resistant ER+ metastatic breast cancer, N550K and M538I. N550K is a well-known activating FGFR2 mutation; M538I stabilizes the active kinase conformation and it has not previously been described in breast cancer. When expressed in ER+ T47D cells, FGFR2 M538I and N550K led to resistance to fulvestrant, and the CDK4/6 inhibitor palbociclib and the combination of the two agents. FGFR2 M538I induced hyperactivity of p-FRS2, p-ERK and p-AKT, which is higher than wildtype FGFR2 and comparable to other known activating mutations N550K and K660N. In addition, overexpression of M538I mutant reduced sensitivity to FGFR inhibitors PD173074 and dovitinib in T47D cells, suggesting M538I is also functionally activating. Due to the hyperactive downstream signaling elicited by the mutation, cells overexpressing FGFR2 M538I achieved optimal growth in the presence of low dose of FGFR inhibitor. Under such conditions, FGFR2 M538I conferred more potent resistance to fulvestrant as compared to wildtype FGFR2. However, drug resistance resulting from M538I mutant can be fully resensitized to fulvestrant and/or palbociclib with high dose of FGFR inhibitors.

In summary, we have identified activating FGFR2 mutations (M538I and N550K) in ER+ breast cancer patients, which may contribute to the development of resistance to SERDs and CDK4/6 inhibitors. Additional FGFR2 mutations have been recently identified in other cohorts of patients with resistant ER+ metastatic breast cancer, suggesting that this may be a clinical mechanism of resistance in some patients. Patients with activating FGFR2 mutations may benefit from the treatment with an FGFR inhibitor in combination with SERDs and CDK4/6 inhibitors.
Advantageous polypharmacology of abemaciclib revealed by omics profiling of CDK4/6 inhibitors

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Small molecule inhibitors of cyclin-dependent kinases (CDK) 4/6 have created new opportunities for the treatment of advanced hormone-receptor positive (HR+) breast cancer and show promise in other malignancies. Three CDK4/6 inhibitors, palbociclib (PD0332991; Ibrance), ribociclib (LEE011; Kisqali), and abemaciclib (LY2835219), which have been approved or are in late stage trials, are reported to be broadly similar although recent data suggest that abemaciclib has distinct single-agent activity in patients and a unique adverse effects profile. Differences in pharmacokinetics and relative potency for CDK4 versus CDK6 are postulated to account for these differences. In this paper, we use molecular and functional profiling by mRNA sequencing, mass spectrometry-based proteomics, and GR-based dose-response assays to obtain complementary views of the mechanisms of action of CDK4/6 inhibitors. We show that abemaciclib, but not ribociclib or palbociclib, is a potent inhibitor of kinases other than CDK4/6, including CDK1/Cyclin B, which appears to cause arrest in the G2 phase of the cell cycle, and CDK2/Cyclin E/A, which is implicated in resistance to palbociclib. Whereas ribociclib and palbociclib induce cytostasis, and cells adapt to these drugs within 2-3 days of exposure, abemaciclib induces cell death and durably blocks cell proliferation. Abemaciclib is active even in retinoblastoma protein (pRb)-deficient cells in which CDK4/6 inhibition by palbociclib or ribociclib is completely ineffective. The degree of polypharmacology of small molecule drugs is increasingly viewed as an important consideration in their design, with implications for efficacy, toxicity, and acquired resistance. In the case of CDK4/6 inhibitors, we propose that abemaciclib polypharmacology elicits unique molecular responses that are likely to be therapeutically advantageous. More generally, we propose that multi-omic approaches are required to fully elucidate the spectrum of targets relevant to drug action in tumor cells. We expect such understanding to assist in stratifying patient populations and ordering sequential therapies when resistance arises.
Title: CDK4/6 inhibition blocks effects of IGFs and insulin in estrogen receptor positive and triple negative breast cancers: Implications for cotargeting IGF1R/IR and CDKs

Deepali Sachdev and Katelyn Hoff. 1Masonic Cancer Center, University of Minnesota, Minneapolis.

Body: IGF and insulin signaling via the type I insulin-like growth factor receptor (IGF1R) and insulin receptor (IR) respectively, are potent activators of PI3K/Akt/mTOR. Drugs targeting IGF1R and the related IR were tested clinically including in combination with mTOR inhibitors. Inhibition of mTOR was not effective as inhibition of mTOR relieved the negative feedback loop regulating levels of the adaptor protein, insulin receptor substrate 1 (IRS-1), that mediates proliferative effects of IGFs and insulin and rapamycin enhanced phosphorylation of Akt. The ribosomal protein S6 kinase (S6K) phosphorylates IRS-1 on serine residues and targets it for proteasomal degradation and this negative feedback regulation is important in attenuating IGF and insulin signaling. Cyclin dependent kinases (CDKs) 4 and 6 are required for cell cycle progression. CDK4/6 inhibitors have recently been approved for treatment of estrogen receptor positive (ER+), Her2- advanced breast cancers and these CDK4/6 inhibitors such as palbociclib block phosphorylation of retinoblastoma (Rb). IGFs and insulin stimulate cell cycle progression and increase cyclin D1 levels in breast cancers. Therefore, we hypothesized that CDK4/6 inhibition can be combined with IGF1R/IR targeting to block mitogenic functions of IGF/insulin signaling in breast cancer as this would not relieve the negative feedback regulation of IGF and insulin signaling. Herein, we analyzed the effect of palbociclib on IGF-I/insulin signaling, Rb phosphorylation and growth of various subtypes of breast cancer cells. Palbociclib blocked growth of both endocrine sensitive and resistant ER+ breast cancers. Further, ER+ parental MCF-7 and T47D cells that respond to hormonal therapy including tamoxifen, a selective estrogen receptor modulator, were more sensitive to palbociclib compared to matched cells with acquired resistance to tamoxifen. Palbociclib blocked IGF-I and insulin stimulated entry into cell cycle leading to G0/G1 arrest in ER+ breast cancer cells. Further, palbociclib also blocked growth and cell cycle progression of triple negative breast cancer (TNBC) cells. Unlike mTOR inhibitors that upregulated IRS-1 levels leading to increased phosphorylation of Akt through IGF1R/IR, palbociclib did not affect IRS-1 levels and did not enhance phosphorylation of Akt in ER+ and TNBC cells. Further, combination of palbociclib with IGF1R inhibitory antibody was more effective in inhibiting growth of ER+ breast cancer cells. Our data show that palbociclib can be a potential therapeutic strategy for TNBC and that combining IGF1R/IR inhibitors with palbociclib may be superior to combining them with mTOR inhibitors for ER+ breast cancer.
2017 San Antonio Breast Cancer Symposium

Publication Number: PD4-04

Title: Combined inhibition of mTOR and CDK4/6 is required for optimal blockade of E2F function and long term growth inhibition in estrogen receptor positive breast cancer

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Body: The cyclin dependent kinase (CDK) – retinoblastoma (RB) -E2F pathway plays a critical role in the control of cell cycle in estrogen receptor positive (ER+) breast cancer. Small molecule inhibitors of CDK4/6 have shown promise in this tumour type in combination with hormonal therapies, reflecting the particular dependence of this subtype of cancer on cyclin D1 and E2F transcription factors. mTOR inhibitors have also shown potential in clinical trials in this disease setting. Recent data has suggested cooperation between the phosphatidylinositol 3-kinase (PI3K) pathway and CDK4/6 inhibition in preventing early adaptation and eliciting growth arrest, but the mechanisms of the interplay between these pathways have not been fully elucidated. Here we show that profound and durable inhibition of ER+ breast cancer growth is likely to require multiple hits on E2F mediated transcription. We demonstrate that inhibition of mTOR using the mTORC1/2 inhibitor vistusertib at 300nM causes a >50% decrease in cyclin D1 protein levels and RB phosphorylation in three cell lines. At these concentrations, vistusertib treatment also elicits marked effects on E2F mediated transcription, causing changes in the mRNA levels of 28 out of 43 (65%) of a selected set of E2F target genes. Combined inhibition of mTOR, CDK4/6 and ER delivers profound and durable regressions in breast cancer cell lines and xenografts (110.2% tumour growth inhibition at day 48). In vivo data show, that over a period of 58 days, tumours failed to re-grow in the presence of the triplet combination compared to either agent alone, suggesting, that the triplet is necessary to maintain growth inhibition. Furthermore, we show that CDK4/6 inhibitor resistant cell lines re-activate the CDK-RB-E2F pathway, but remain sensitive to mTOR inhibition (EC50 52.7 nM in parental cells vs 39.6-73.3 nM in a number of palbociclib resistant cell populations), suggesting that mTORC1/2 inhibitors may represent an option for patients that have relapsed on CDK4/6 therapy. A Phase I study (PASTOR) combining the dual TOR kinase inhibitor Vistusertib with Palbociclib, and Fulvestrant is underway to explore safety and efficacy of the triplet combination in patients with metastatic breast cancer.
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Title: Patterns of genomic alterations in ER-positive advanced breast cancer patients treated with CDK4/6 inhibitors

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Body: Background: Cyclin D kinase inhibitors (CDK-is) have shown clinical efficacy in estrogen receptor (ER)-positive metastatic breast cancer (MBC) when combined with aromatase inhibition or estrogen receptor (ER) antagonism. Despite the benefit of this approach, clinical resistance develops sometimes early in the treatment without any response to endocrine therapy (primary endocrine resistance) or after initial response (secondary resistance) in all patients in the metastatic setting and the molecular basis for this resistance are still largely unknown. We evaluated the pattern of genomic alterations in circulating cell-free tumor DNA (ctDNA) analysis of metastatic breast cancer patients with ER-positive tumors treated with palbociclib combined with either letrozole or fulvestrant and progressing during therapy.

Methods: We conducted a retrospective study of patients with ER-positive MBC who had longitudinal assessment of their disease by ctDNA analysis. The plasma-based assay was performed utilizing Guardant360 (Guardant Health, CA), a digital NGS technology to sequence a panel of > 50 cancer genes. After tabulating number of genomic alterations detected for every patient at baseline and after CDK-i therapy, analysis was performed to identify molecular profile changes in the entire population and in individuals with early progression of disease (<6 months).

Results: We analyzed data of 15 ER-positive MBC patients: 8 patients received fulvestrant/palbociclib and 7 received letrozol/palbociclib. The most common mutations before CDK-i therapy were: PIK3CA (16%), TP53 (16%), ESR1 (13%), KIT (9%), EGFR (3%), APC (3%), ERBB2 (3%), MYC (3%), PTEN (3%), RB1 (3%). After therapy with CDK-i the pattern of mutations showed stable and persistent incidence of PIK3CA, TP53 and ESR1. However, new mutations where identified: FGFR1 (6%), IDH (2%), BRCA1 (2%), BRCA2 (2%), CCNE (2%), CCND1 (2%), RAF (2%), AR (2%), ALK(2%). Also, the pattern of gene amplifications presented an increased rate of MYC and FGFR1 amp. Patients with progression of disease before 6 months of CDK-i therapy presented baseline higher number and variation of mutations compared to patients with disease controlled beyond 6 months of therapy.

Conclusion: Longitudinal assessment with ctDNA analysis suggest that a genomic alteration landscape consisting of persistent detection of driver and acquired mutations along with emergent new abnormalities in regulatory genes could potentially be related to primary or secondary resistance to CDK-Is in ER+ MBC patients. Future investigation of these alterations should be conducted.
Title: First-line ribociclib + letrozole in hormone receptor-positive, HER2-negative advanced breast cancer: Efficacy by baseline circulating tumor DNA alterations in MONALEESA-2

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Body: Background: The addition of first-line ribociclib (RIB; cyclin-dependent kinase 4/6 inhibitor) to letrozole (LET) significantly improved progression-free survival (PFS) compared with placebo (PBO) + LET in patients (pts) with hormone receptor-positive (HR+), human epidermal growth factor receptor 2-negative (HER2−) advanced breast cancer (ABC) in the Phase III MONALEESA-2 study. Identifying biomarkers that predict response to treatment remains a key challenge in pts with HR+ ABC. Here we analyze results from MONALEESA-2 by molecular alterations detected in circulating tumor DNA (ctDNA) at baseline, including PIK3CA mutations and other alterations considered to be important in HR+ ABC.

Methods: Postmenopausal women (N=668) with HR+, HER2− ABC who had not received any prior therapy for ABC were randomized 1:1 to RIB (600 mg/day; 3-weeks-on/1-week-off) + LET (2.5 mg/day; continuous) or PBO + LET. The primary endpoint was PFS. Biomarker analysis of the ctDNA mutation profile was an exploratory endpoint. Plasma samples for ctDNA analysis were collected at baseline and end of treatment. ctDNA was analyzed using next-generation sequencing with a targeted panel of ~550 genes.

Results: Baseline ctDNA was successfully sequenced in 494 pts (RIB + LET: n=212; PBO + LET: n=215); 67 (14%) of 494 pts were removed from the analysis due to limited tumor DNA in circulation. 427 (86%) pts had ≥1 alteration, including 1,573 mutations, 513 short insertions/deletions, 166 amplifications, and 8 translocations. Alterations (frequency) were commonly observed in the following genes: PIK3CA (33%), TP53 (12%), ZNF703/FGFR1 (5%), and ESR1 (4%), and in genes involved in receptor tyrosine kinase (RTK) signaling (12%). RIB + LET treatment benefit was consistent in pts with wild-type (WT) and altered PIK3CA, and in pts with WT and altered TP53 (Table). RIB + LET improved PFS regardless of RTK or ZNF703/FGFR1 alterations. However, there was a weak trend for increased benefit in pts with WT vs altered RTK genes and in pts with WT vs altered ZNF703/FGFR1 genes. These results should be interpreted with caution due to the small number of pts with these alterations. There were too few ESR1 alterations for firm conclusions to be drawn.

<table>
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<th>Gene(s)</th>
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<th>Hazard ratio (95% confidence interval)</th>
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<td></td>
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<td>PBO + LET</td>
<td>RIB + LET</td>
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<tr>
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<td>22/32</td>
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<tr>
<td>RTK</td>
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</table>

**Conclusions:** Consistent RIB + LET treatment benefit was observed compared with PBO + LET, irrespective of the status of baseline ctDNA biomarkers.
Title: Genomic landscape of breast cancers with FGFR1 amplification and FGFR1/CCND1 co-amplification revealed by targeted capture next generation sequencing

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Body: Background: FGFR1 amplification (amp) occurs in ~15% of breast cancers (BC) and associates with poor prognosis and resistance to endocrine therapy. CCND1 regulates cell cycle progression and is amplified in 15-20% of BC. Co-amp of FGFR1 occurs in 30-40% of CCND1amp tumors, suggesting the possibility of oncogene cooperativity. CDK4/6 inhibitors, which block the action of cyclin D1/CDK4 complexes at the G1-to-S transition, are approved for treatment of ER+ BC and FGFR inhibitors are in early phase clinical trials.

Results: Between 11/2013 and 03/2017, 191 BCs from 188 patients with metastatic (M) or refractory locoregional recurrent (RLRR) BC at Vanderbilt (VICC) were profiled by targeted next gen sequencing (Foundation One™). These are included within the 2131 publicly available BC sequencing results in GENIE (Foundation One™ and MSK-IMPACT). Among the GENIE cohort, rates were: FGFR1amp 7% (n=156), CCND1amp 12% (n=261) and CCND1/FGFR1co-amp 3% (n=58). Additional cases showed FGFR1 missense mutations (n=16) and deep deletions (n=5). When the analysis was limited to the VICC cohort allowing restriction to ER+ BC, FGFR1amp (16%) and CCND1amp (23%) rates are similar to rates in primary BC in TCGA (13% FGFR1 [p = 0.44] and 19% CCND1 [p = 0.24]). In GENIE, the most frequent co-mutations in FGFR1amp tumors were TP53 (31%), PIK3CA (21%), GATA3 (13%), CDH1 (11%) and MAP3K1 (10%). However, TP53 and PIK3CA mutations were less common among FGFR1amp tumors than FGFR1non-amp cases (p <0.001). FGFR1amp was associated with 11q13 amp (GCND1, FGF3/4/19) in 35% (p<0.001), 20q,13 amp (GNAS, AURKA) in 27% (p=0.01) and 20q11 amp (SRC) in 15% (p=0.001). ESR1 mutations were enriched among CCND1amp tumors (p<0.001) and ARID1A mutations among FGFR1/CCND1co-amp tumors (p 0.048). Among tumors with FGFR1amp, TP53 mutations were mutually exclusive of ESR1 mutations (p>0.016). Histopathologic correlation on tumors from our institution show a majority of FGFR1 and/or CCND1amp BC (64%) were ER+/HER2–; 33% of ER+ FGFR1amp tumors were PR–. Distinctive histologic features associated with FGFR1 and/or CCND1amp were lobular histology (17%) and neuroendocrine differentiation (14%), 0-10%TILs (94%) and high proliferative rate (46%).

Conclusion: FGFR1amp and CCND1amp rates in TCGA are similar to those seen in MBC/LRRBC (GENIE) suggesting FGFR1 can function as both a driver mutation and de novo mechanism of endocrine resistance early in tumorigenesis. Frequent co-amp with CCND1 and lower rates of TP53 and PIK3CAmut also support a driver role for FGFR1amp and FGFR1/CCND1co-amp. The observation of neuroendocrine features in a subset of these tumors suggests lineage plasticity. This may be a consequence of genomic alterations promoting anti-estrogen resistance and is consistent with recently published BC outcome data associating neuroendocrine differentiation with higher grade ER+ tumors, frequent 8p amp, which includes FGFR1, and worse disease-free and overall survival. The frequency of FGFR1amp suggests genotype specific trials with FGFR inhibitors would be highly feasible. Whether FGFR1/CCND1 co-amplified tumors are candidates for treatment with a combination of FGFR and CDK4/6 inhibitors requires further investigation.
Title: A microenvironment secretome screen reveals FGF2 as a mediator of resistance to anti-estrogens and PI3K/mTOR pathway inhibitors in ER+ breast cancer

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Body: Despite the clinical success of anti-estrogen therapies, phosphatidylinositol 3-kinase inhibitors (PI3Ki), and mechanistic target of rapamycin complex I inhibitors (mTORC1i) for the treatment of patients with ER+ breast cancer, disease recurrence and progression are common. We found that a tumor transcriptional profile reflecting high stromal fibroblast content was associated with poor outcome in 3 cohorts of patients with ER+ breast cancer. We hypothesized that individual factors in the tumor microenvironment (TME) significantly contribute to drug resistance.

To test this hypothesis, we screened 297 recombinant secreted proteins for ability to confer resistance to the anti-estrogen fulvestrant in MCF-7 and T47D ER+ breast cancer cells. Screen results were validated, and expansion screening included the anti-estrogen tamoxifen, the PI3Ki pictilisib, and the mTORC1i everolimus in 4 cell lines. To identify hits are most likely to be relevant to ER+ breast cancer, a bioinformatics filter was developed utilizing gene and protein expression in human tissues relevant to the TMEs of ER+ breast cancer. After filtering, the top screening hit was fibroblast growth factor 2 (FGF2), which confers resistance to anti-estrogens, PI3Ki, and mTORC1i, and is highly expressed in tissues and cell types associated with ER+ breast cancer. FGF2 did not rescue cells from the CDK4/6i palbociclib or the DNA-damaging agent doxorubicin, demonstrating pathway selectivity in the rescue phenotype. FGF2 rescued cells from anti-estrogen-, PI3Ki-, and mTORC1i-induced apoptosis and cell cycle arrest via activation of FGFR signaling through FRS2a, MEK1/2, ERK1/2, and downstream upregulation of cyclin D1 and degradation of Bim. FGF2-mediated anti-cancer effects were abrogated by co-treatment with the FGF2-neutralizing antibody GAL-F2, the pan-FGFR inhibitor PD-173074, the MEK inhibitor trametinib, or palbociclib. Cell cycle- and apoptosis-specific effects of FGF2 were abrogated by RNAi targeting cyclin D1 and Bim, respectively.

We generated a transcriptional signature of FGF2 response by RNA-seq of fulvestrant-treated MCF-7 and T47D cells treated +/- FGF2. In 3 cohorts of patients with ER+ breast cancer, a signature of FGF2 signaling was significantly associated with poor prognosis and predictive of anti-estrogen resistance, including in a multivariate analysis including age, tumor grade, tumor stage, and FGFR amplification status. Finally, the therapeutic potential of targeting FGF2 was confirmed in 3 mouse models of ER+ breast cancer: 1) FGF2 rescue MCF-7 xenografts from fulvestrant; 2) GAL-F2 synergized with fulvestrant to suppress growth of 59-2-HI murine mammary adenocarcinomas that recruit FGF2-secreting stroma; 3) GAL-F2 synergized with fulvestrant to induce regression of HCI-003 patient-derived xenografts. Therapeutic effects coincided with increased tumor cell apoptosis and decreased proliferation, but not changes in tumor vasculature. These findings warrant consideration of FGF2 as a novel therapeutic target in ER+ breast cancer.
Body: Background
Invasive Lobular Carcinoma (ILC) is an understudied subtype of breast cancer that requires novel therapies in the advanced setting. Distinctive properties of ILC include growth patterns, metastatic behavior, and receptor status (almost universally estrogen receptor (ER) positive). Our lab recently generated six long-term estrogen deprivation (LTED) models of ILC cells and performed RNA-Sequencing to identify differentially expressed genes compared to their parental cells cultured with estrogen. We overlapped these results with a previously published microarray dataset and found that FGFR4 is the most consistently overexpressed gene in the setting of acquired resistance to endocrine therapy in ILC cells.

Hypothesis
FGFR4 is an important mediator of resistance to endocrine therapy in ILC.

Methods
To study the role of FGFR4 in vitro, we used multiple shRNAs and specific small molecule inhibition for growth assays of ILC cells. To study the role of FGFR4 in de novo resistance to endocrine therapy, we collected 129 well curated ER+ ILC tumor specimens and performed gene expression analysis on the pre-treatment samples using a custom NanoString panel. To study the role of FGFR4 in acquired resistance, we collected over 50 pairs of primary-metastatic ER+ tumors and performed exon capture based RNA-Sequencing.

Results
FGFR4 inhibition decreases parental and LTED ILC cell growth in classic 2D conditions, in the setting of ultra-low attachment, and in colony formation assays. The LTED cells, with higher FGFR4 expression, are more sensitive to its inhibition. For the parental cells, combination FGFR4 and ER-targeting drugs results in synergistic decreases in growth. In our database of primary ILC clinical samples, increased expression of FGFR4 is predictive of shorter time to distant recurrence. For our collection of 50 paired, primary-metastatic ER+ tissues, FGFR4 expression increases on average >2.5 fold in the metastatic setting, with large gains even in ductal carcinoma cases. Finally, in analyzing recently published cohorts of metastatic tumors, there is a significant enrichment of hotspot FGFR4 mutations in tumors originating in the breast, with >2% of metastatic ILC tumors containing such a mutation.

Conclusion
FGFR4 may play an important role in both acquired and de novo resistance to endocrine therapy in ILC.
Title: FGFR1 / ZNF217 copy numbers and outcome in patients with node positive, HR+/Her2- early breast cancer: A genomic analysis of PACS04 trial

Body: Purpose: There is a need to identify which patients present a high risk of relapse after optimal adjuvant therapies, in order to better define which population of early breast cancer patients is eligible to new drugs. Previous studies have shown that gene copy numbers drive breast cancer progression. In the BIG1-98 trial, FGFR1 (8p11) and ZNF217 (20q13) amplifications were associated with poor outcome in patients with HR+/Her2- early breast cancers. In the present study, we aim to validate these findings using retrospective analysis of samples prospectively collected in a randomized trial (PACS04).

Patients and methods: Tumor DNA was extracted from FFPE samples obtained from patients included in PACS04 trial. This trial compared FEC to ED75 in patients with node positive, early breast cancer and included 3000 patients between 2001 and 2004. The biomarker study focuses on HR+/Her2- breast cancer. 500 samples were collected and hybridization was performed on 390. DNA copy numbers were assessed on 900 genes using Oncoscan FFPE assay kit. Prognostic value of FGFR1 (8p11) and ZNF217 (20q13) copy numbers was assessed in a Cox model that included prognostic parameters. Prognostic value was assessed using CNA as continuous variable, and with a predefined cut-off (CN>=3). Metastases-free survival (MFS) was chosen as endpoint for this biomarker study.

Results: Tumor copy numbers alterations were obtained in 390 patients with HR+/Her2-, node positive, early breast cancer. 31% of the patients presented more than 3 lymph node involved, and 25% a poorly differentiated tumor. The median tumor size was 20 mm. 120 (30%) and 112 (28%) patients presented FGFR1 and/or ZNF217 gene gain with a cut-off for amplification predefined at 3 copies. When assessed as continuous variable, FGFR1 (HR: 1.02, 95%CI:1.007-1.04, p=0.0045) and ZNF217 (HR: 1.011, 95%CI:1.003-1.01, p=0.006) copy numbers were associated with MFS. The 10 years MFS rates were 68% (95%CI: 59-78%) and 85% (95%CI: 81-91%) in patients with FGFR1-gained and FGFR1-normal tumors respectively (HR: 2.51, 95%CI:1.56-4.01, p=0.0001). The 10 years MFS rates were 72% (95%CI:65%-83%) and 83% (95%CI: 77-87%) in patients with ZNF217-gained and ZNF217-normal tumors (HR: 1.79, 95%CI:1.12-2.86, p=0.013). In a multivariate analysis, FGFR1-gain (HR: 2.45, 95%CI:1.42-4.22, p=0.0012) and ZNF217-gain (HR:1.78, 95%CI:1.00-3.17, p=0.049) were associated with a higher likelihood of metastases and/or death. The 10 MFS rate was 88% (95%CI: 83-94%) for patients who presented FGFR1- and ZNF217-normal patients (n=191), and 71% (95%CI: 66-76%) in patients presenting FGFR1 and/or ZNF217 gene gain.

Conclusion: Using samples prospectively collected in a randomized trial, this study validates the prognostic value of FGFR1- and ZNF217- copy numbers in patients who present HR+/Her2- early breast cancer. Exploratory analyses on copy number alterations, together with targeted sequencing are ongoing and will be presented.
Title: Copy-number and targeted sequencing analyses to identify distinct prognostic groups: Implications for patient selection to targeted therapies amongst anti-endocrine therapy resistant early breast cancers

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Body: Hormone receptor positive breast cancer remains an ongoing therapeutic challenge. Despite optimal anti-endocrine therapies, most breast cancer deaths follow a diagnosis of early luminal cancer. Data describing molecular events in breast cancer has yet to be translated into actionable information to inform medical management and benefit patients. To understand the impact of multiple aberrations in the context of current therapy, we assessed the prognostic ability of genomic signatures as a putative stratification tool to targeted therapies. The analysis was performed based on an a priori hypothesis relating to molecular pathways which might predict response to targeted therapies currently under evaluation in late-stage clinical trials. In a case-control fashion, 420 patients from the Tamoxifen vs Exemstane Adjuvant Multinational Trial (TEAM) pathology cohort, were analysed to determine the prognostic, ability for these mutational and copy-number biomarkers representing the CCND/CDK, FGFR/FGF and AKT/PIK3CA to inform potential response to therapies targeting these pathways. Copy number analysis was performed using the Affymetrix Oncoscan™ Assay. Targeted sequencing was performed in a subset of samples for genes based on signaling cassettes mined from the ICGC. Pathways were identified as aberrant if there were copy number aberrations (CNAs) and/or mutations in any of the predetermined pathway genes: 1) CCND1/CCND2/CCND3/CDK4/CDK6 2) FGFR1/FGFR2/FGFR2/FGFR4 and 3) AKT1/AKT2/PIK3CA/PTEN. Kaplan Meier and log rank analyses were used for DRFS between groups. Hazard ratios were calculated using the Cox proportional hazard models adjusted for age, tumour size, grade, lymph node and HER2 status. 390/420 samples passed informatics QC filters. For the CCND/CDK pathway, patients with no CNA changes experienced a better DRFS (HR=1.94, 95% CI 1.45-2.61, p< 0.001). For the FGFR/FGF pathway, a similar outcome is seen among patients without CNAs (HR = 1.43, 95% CI 1.07-1.92 p=0.017). For AKT/PIK3CA, a decrease in DRFS was seen in those with aberrations (H=1.34, 95% CI 1.00-1.81, p=0.053). We demonstrated that CNAs of genes within CDK4/CCND, PIK3CA/AKT and FGFR pathways are independently linked to high risk of relapse following endocrine treatment. In this way, improving the clinical management of early breast cancers could be made, firstly by identifying those patients for whom current endocrine therapies are sufficient, thus reducing unnecessary treatment; and secondly, identifying those patients who are at high-risk for recurrence despite optimal endocrine therapy and the linking molecular features driving these cancers to treatment with targeted therapies.
Title: Clustering of activated proteins of the PI3K and MAPK pathways distinguishes ER+/HER2- primary breast cancer patients with preferential tamoxifen benefit

Dinja T Kruger1,2, Karin Beelen3, Mark Opdam2, Joyce Sanders2, Vincent van der Noort2, Epie Boven1 and Sabine C Linn2,4. 1VU University Medical Center, Amsterdam, Netherlands; 2The Netherlands Cancer Institute, Amsterdam, Netherlands; 3Renier de Graaf Groep, Delft, Netherlands and 4University Medical Center Utrecht, Utrecht, Netherlands.

Body: Background
Several (activated) proteins of the phosphatidylinositol-3-kinase (PI3K) and/or mitogen-activated protein kinase (MAPK) pathway have been analyzed for an association with adjuvant tamoxifen benefit in estrogen receptor-positive (ER+), HER2-negative (HER2-) breast cancer. Currently, no single protein has yielded convincing results allowing clinical implementation. Combining activated proteins could provide more robust predictive potential. We used unsupervised hierarchical clustering of 7 activated proteins downstream in these pathways, discriminated by expression profile, to analyze adjuvant tamoxifen benefit in ER+/HER2-postmenopausal breast cancer patients. Further, we developed a classification tool based on the generated heatmap groups by pathway activation status to categorize future patients into sensitive for or resistant to adjuvant tamoxifen.

Methods
Primary tumor blocks from 489 ER+/HER2- (stage I-III) postmenopausal patients previously randomized between tamoxifen (1 to 3 years) vs no adjuvant therapy (IKA trial 1982-1993; Beelen et al, Breast Cancer Res, 2014) were used for immunohistochemistry on tissue microarrays. Median follow-up of the patients without a recurrence event was 8.5 years. Scoring of PTEN, p-AKT(Thr308), p-AKT(Ser473) and p-p70S6K(Thr389) was by cytoplasmic intensity (0-3), p-4EBP1(Ser65) and pERK1/2(Thr202/204) by percentage of tumor cells with positive nuclear staining and p-S6RP(Ser235/236) by the percentage of tumor cells with positive membranous staining. Unsupervised hierarchical clustering was performed on tissue from 293 patients with informative data on all 7 proteins. Cox models were used to assess tamoxifen benefit (defined as the Hazard ratio (HR) of tamoxifen treatment vs no adjuvant therapy for recurrence-free interval) and to compare this benefit between subgroups generated by clustering as well as generated by the classification tool. Cox models included covariates (age, histological grade and subtype, tumor size, PR status) and were stratified for lymph node status.

Results
Two distinct groups were identified based on hierarchical clustering: one with preferentially activated pathway markers (A) and one with relatively no activation (N). Patients in group N derived significant benefit from tamoxifen (multivariable HR 0.28, 95% CI 0.14 – 0.56, p = 0.000324), while patients from group A had no benefit (multivariable HR 1.57, 95% CI 0.44 – 5.56, p = 0.482), p for interaction 0.018. Contradictory, patients in group A had a better prognosis compared to those in group N (multivariable HR 0.17, 95% CI 0.04 – 0.7, p = 0.014). The classification tool was also successful in differentiating ER+/HER2-patients with or without tamoxifen benefit (p for interaction = 0.016).

Conclusions
Hierarchical clustering of PI3K/MAPK pathway proteins is able to differentiate patients with adjuvant tamoxifen benefit and patients who would potentially need alternative treatment, for instance with inhibitors of the PI3K or MAPK pathway. The classification tool distinguishing patients with and without tamoxifen benefit should be validated in an independent cohort of postmenopausal patients with primary ER+/HER2- breast cancer.
Title: Estrogen receptor-negative breast adenomyoepitheliomas are driven by co-occurring HRAS hotspot and PI3K pathway gene mutations: A genetic and functional analysis

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Body: Introduction: Adenomyoepithelioma (AME) of the breast is a rare biphasic tumor, characterized by epithelial and myoepithelial differentiation. Although AMEs have an indolent clinical course, a subset may progress to carcinoma and metastasize. We sought to define the mutational landscape of AMEs and investigate the functional impact of recurrent pathogenic mutations identified in these tumors.

Methods: Thirty-one AMEs were subjected to whole-exome sequencing (WES, n=8) or massively parallel sequencing targeting all coding regions of 410 key cancer genes and intronic and regulatory regions of selected genes (n=23). Somatic genetic alterations were defined using state-of-the-art bioinformatics algorithms. In an additional set of 12 AMEs, Sanger sequencing analysis of HRAS, PIK3CA and AKT1 was performed. Non-tumorigenic estrogen receptor (ER)-negative mammary epithelial cells (i.e. MCF10A, MCF10A with a PIK3CA H1047R mutation knock-in and MCF12A) were utilized for functional studies using both conventional monolayer and three-dimensional (3D) culture assays.

Results: 27 (63%) and 16 (37%) AMEs were ER-positive and ER-negative, respectively. ER-negativity was significantly associated with histologic features predictive of a more aggressive behavior, with a higher number of mutations and copy number alterations, and with a distinct mutational profile as compared to ER-positive AMEs. Of the 27 ER-positive AMEs, 12 cases (44%) harbored PIK3CA hotspot mutations, and 5 PIK3CA wild-type cases displayed E17K AKT1 hotspot mutations. By contrast, of the 16 ER-negative AMEs, 9 (56%), 9 (56%) and 3 (19%) harbored HRAS, PIK3CA (mostly E545K and H1047R hotspots) and PIK3R1 mutations, respectively. Strikingly, all HRAS mutations were restricted to ER-negative AMEs, affected the hotspot codon Q61 (Q61R/K), and all but one co-occurred with PIK3CA or PIK3R1 mutations. In addition, HRAS Q61 hotspot mutations were significantly associated with necrosis (p=0.01) and high mitotic rates (p=0.03). CDKN2A homozygous deletions were also detected only in ER-negative AMEs (19%) and found to be significantly associated with progression to carcinoma (p=0.001). Forced expression of HRAS Q61R in MCF10A and MCF12A cells resulted in i) increased proliferation and transformation, ii) an irregular growth pattern in 3D organotypic cell cultures, iii) partial loss of the epithelial phenotype, and iv) acquisition of myoepithelial differentiation, which was more overt in PIK3CA-mutant MCF10A cells. HRAS Q61R induced hyperactivation of the PI3K pathway, but both PI3K and MAPK pathways likely contributed to the RAS-mediated proliferation, which was completely arrested by combined AKT and MEK inhibition.

Conclusion: AMEs are phenotypically and genetically heterogeneous. Whilst pathogenic mutations in PI3K pathway-related genes occur across the spectrum of lesions, HRAS Q61 hotspot mutations are restricted to ER-negative AMEs. Our genomic and functional analyses indicate that HRAS Q61 mutations are driver events in the pathogenesis of ER-negative AMEs and, in conjunction with mutant PIK3CA, may lead to the acquisition of myoepithelial differentiation in breast epithelial cells.
Title: GDC-0077 is a selective PI3Kalpha inhibitor that demonstrates robust efficacy in PIK3CA mutant breast cancer models as a single agent and in combination with standard of care therapies

Rebecca Hong¹, Kyle Edgar¹, Kyung Song¹, Schmidt Steven¹, Amy Young¹, Patricia Hamilton¹, Alfonso Arrazate¹, Cecile De La Cruz¹, Connie Chan¹, Jodie Pang¹, Laurent Salphati¹, Marcia Belvin¹, Michelle Nannini¹, Steven Staben¹, Lori Friedman¹ and Deepak Sampath¹. ¹Genentech, South San Francisco, CA.

Body: The phosphatidylinositol 3 kinase (PI3K)/AKT/mechanistic target of rapamycin (mTOR) signaling pathway is a major regulator of tumor cell growth, proliferation and survival. Dysregulation of the PI3K/AKT/mTOR signaling pathway through multiple different mechanisms has been described in solid tumor malignancies, including activating and transforming mutations and amplification of PIK3CA, that encodes the p110alpha subunit of PI3K. Indeed, PIK3CA hotspot mutations are highly prevalent in breast cancer, occurring in approximately 40% of HR+ tumors. The clinical candidate GDC-0077 is a potent inhibitor of PI3Kalpha (IC50 = 0.038 nM) and exerts its activity by binding to the ATP binding site of PI3K, thereby inhibiting the phosphorylation of PIP2 to PIP3. Biochemically, GDC-0077 is >300-fold more selective for PI3Kalpha over the other class I PI3K isoforms (beta, delta, and gamma) and >2000-fold more selective over PIK family members. Furthermore, GDC-0077 is more selective for mutant versus wild-type PI3Kalpha in cell based assays. The improved biochemical selectivity of GDC-0077 relative to PI3Kdelta translated in human CD69+ B-cells, which are primarily dependent on PI3Kdelta for proliferation and survival, and were more sensitive (based on reduction of cell number) to the PI3Kalpha/delta selective inhibitor taselisib (GDC-0032) than to GDC-0077. Mechanism of action (MOA) studies indicate that GDC-0077 selectively degrades mutant PI3Kalpha in a proteasome-dependent fashion resulting in reduction of PI3K pathway activity biomarkers such as pAKT and pPRAS40, inhibition of cell proliferation, and increased apoptosis in human PIK3CA-mutant breast cancer cell lines to a greater extent when compared to PIK3CA wild-type cells. In vivo, oral daily treatment of patient-derived PIK3CA-mutant breast cancer xenograft models with GDC-0077 resulted in tumor regressions, induction of apoptosis, and a reduction of pAKT, pPRAS40, and pS6RP in a dose-dependent fashion. In vivo efficacy in a PIK3CA-mutant human breast cancer xenograft model was also improved when GDC-0077 was combined with therapies for hormone-receptor positive (HR+) breast cancer such as anti-estrogens (fulvestrant) or a CDK4/6 inhibitor (palbociclib). Collectively, preclinical studies provide rationale for evaluating GDC-0077, a PI3Kalpha selective inhibitor that degrades mutant p110alpha protein, as a single agent and in combination with endocrine and targeted therapies that may provide additional benefit to patients with locally advanced or metastatic hormone receptor+ breast cancers that harbor PIK3CA mutations.
Title: A tale of two pathways: Mutations in PI3K pathway in TNBC patients matter for the oncogenic cooperation with DNA damage repair pathway

Nandini Dey¹, Casey Williams¹, Amy Krie¹, Jessica Klein¹, Kirstin Williams¹, Jennifer H Carlson¹, Pradip K De¹ and Brian Leyland-Jones¹. ¹Avera Center for Precision Oncology, Avera Cancer Institute, Sioux Falls, SD.

Body: Background: Mutations guide targeted therapy in the personalized medicine. In the opening chapter of our recently edited book (Dey et al., 2016), Prof. L. Cantley elegantly elucidated the basic signaling of the PI3K pathway in cancers. Mutations in the PI3K pathway are not only common and subtype-specific in BC but are also contextual. Alterations in DNA damage repair (DDR) pathway involving HRD (Homologous Recombination Defect) genes are one of the important contextual events of the upregulation of the PI3K pathway (De et al., 2016). Aim: Here we interrogated the contextuality of alterations of the PI3K and DDR pathway genes in our Avera patients. The mechanism of contextual cooperation between the pathways was experimentally validated. Methods: We examined mutation profile (FoundationOne) of our patients (Avera Cancer Institute) and patients from the TCGA data (cBioPortal). We validated the cooperation of the two pathways experimentally by the synergy model of mutation-specific drugs; PI3K-PTEN-mTOR pathway inhibitor(s) and PARP inhibitor(s) using TNBC model. Results: We analyzed alterations of 17 and 12 genes of the PI3K and DDR pathways respectively in subtypes of BC. In luminal A and HER2-enriched (TCGA, Nature 2012), the alteration of PIK3CA reached 49 % and 47% as compared to 37 % in luminal B and 25% in basal-like. In the basal-like/TNBC subtype (cBioPortal) 12 DDR pathway genes (CHEK1/2, RAD51, BRCA1/2, MLH1, MSH2, ATM, ATR, MDC1, PARP1, FANCF) were altered in 90.1 % of cases, and 17 PI3K pathway genes were altered in 88.9 % of cases. Our ER+ve patients presented a diverse variety of PIK3CA mutations (E545K, E545A, E545G, E542K, E453K, E762K, E365K, N345K, C420R, E81K, Q546R, C420R, E726K, E81K, E970K, H1047R, H1047L P104L, P539R, G106R,G1049R, R93Q,N345T, V105_E109>E, L113del, K111del) as compared to a less diverse type of PIK3CA mutations (Amplification, E542K, H1047R) in our TNBC patients. In our TNBC patients, the predominant type of mutation in PI3K pathway genes was found in PTEN consisting of Y68C, Y180*, loss, loss exons 1-5, and deletion exon1. The other most common mutation found in TNBC patients was in TP53 (>80%) and somatic BRCA1/2 (~15%) genes. The interaction between the two pathways was evaluated using the mostly altered oncogenes and tumor suppressor genes (PTEN, AKT1/2, TSC1/2, mTOR, RICTOR, RHEB, BRCA1/2, ATM, ATR, FANCF) applying STRING10 to test the association at the highest 0.900 confidence views. Finally, we experimentally validated the contextual synergy of 2 pathways by demonstrating that a node-specific inhibition of the PI3K-mTOR pathway by GDC-0980 in the presence of carboplatin resulted in (1) an enhanced impairment of DSB repair and (2) a subsequent sensitization to PARPi (i). This effect occurred simultaneously with the inhibition of classic PI3K-mTOR survival signal(s) which induced a robust antiproliferative/proapoptotic effect even in BRCA-competent TNBC cells. The absence of PTEN, on the other hand, sensitized TNBC cells to PARPi in the presence of carboplatin, an effect more pronounced in BRCA-loss. Conclusion: Our data showed that the PI3K pathway cooperates with the DDR pathway in the breast oncogenesis especially basal-like and TNBC.
2017 San Antonio Breast Cancer Symposium

Title: Final results of NeoMONARCH: A phase 2 neoadjuvant study of abemaciclib in postmenopausal women with hormone receptor positive (HR+), HER2 negative breast cancer (BC)

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Body: Background: Abemaciclib is a selective oral inhibitor of CDK4 and CDK6. Dosed on a continuous schedule, abemaciclib showed evidence of antitumor clinical activity in patients (pts) with metastatic BC in monotherapy or in combination with non-steroidal aromatase inhibitor (NSAI) or fulvestrant. NeoMONARCH (NCT02441946) is a Phase 2 trial in women with stage I-IIIB, HR+/HER2- BC evaluating neoadjuvant treatment with abemaciclib + anastrozole, ANZ. As previously reported, NeoMONARCH met its primary endpoint showing abemaciclib, alone or in combination with ANZ, significantly reduced Ki67 expression compared to ANZ alone after 2 weeks (wks) of treatment. Final results are presented here.

Methods: 223 pts were randomized (1:1:1) and treated for 2 wks with abemaciclib (150 mg PO Q12H) + ANZ (1 mg PO QD), abemaciclib alone, or ANZ alone. Then, all pts were treated for 14 wks with the combination. Pts were treated with loperamide (2 mg PO Q12H) for 4 wks while receiving abemaciclib. Tumor biopsy was collected at baseline, Wk 2 and Wk 16. Blood samples were collected through Cycle 5 to measure abemaciclib and ANZ concentrations. Primary objective was Ki67 change from baseline to Wk 2. Secondary objectives evaluated after Wk 16 of treatment, were radiologic, pathological and clinical responses, safety, and pharmacokinetics (PK). Exploratory objectives included the mutational analysis of PIK3CA and ESR1 at baseline.

Results: Table 1 shows subgroup analyses of percent change in Ki67 from baseline to Wk 2 by disease stage, baseline lymph node (LN) involvement, tumor grade, and tumor size in Ki67 evaluable (KE) population (baseline Ki67 ≥ 5%) comparing combination to ANZ alone. Data for abemaciclib arm will be shown at the meeting. 185 pts completed treatment. At the end of treatment, response rates were radiologic 46.4% (all pts), and caliper 53.6% (all pts), and pCR 3.7% (pts who completed BC surgery assessment). Ki67 end of treatment analysis in 138 pts will be presented. The most common adverse events, all pts, were diarrhea (61.4%; G3: 4.9%), constipation (43.5%; G3: 1.8%), and nausea (41.7%; G3: 2.2%). Treatment discontinuation due to AEs was low (7.6%). Results of PIK3CA and ESR1 mutational analysis, and PK will be presented.

Subgroup analyses at Wk 2 of KE population

<table>
<thead>
<tr>
<th></th>
<th>Combination</th>
<th>ANZ</th>
<th>Subgroup treatment comparison</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>GM % Change</td>
<td>GM % Change</td>
<td>GMR (95% CI)</td>
</tr>
<tr>
<td>KE population</td>
<td>59 -92.86</td>
<td>56 -62.78</td>
<td>0.19 (0.13, 0.28)</td>
</tr>
<tr>
<td>Disease Stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I/II</td>
<td>51 -92.56</td>
<td>46 -65.84</td>
<td>0.22 (0.14, 0.34)</td>
</tr>
<tr>
<td>III</td>
<td>7 -95.28</td>
<td>8 -54.91</td>
<td>0.10 (0.04, 0.27)</td>
</tr>
<tr>
<td>Baseline LN Involvement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>29 -93.18</td>
<td>26 -62.21</td>
<td>0.18 (0.11, 0.31)</td>
</tr>
<tr>
<td>Yes</td>
<td>29 -92.75</td>
<td>28 -69.02</td>
<td>0.23 (0.13, 0.42)</td>
</tr>
<tr>
<td>Tumor Grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor Size</td>
<td>1 or 2</td>
<td>33</td>
<td>-92.88</td>
</tr>
<tr>
<td>----------------------------------</td>
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</tr>
<tr>
<td></td>
<td>3</td>
<td>16</td>
<td>-92.79</td>
</tr>
<tr>
<td><strong>Tumor Size</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 2 cm</td>
<td>9</td>
<td>-93.25</td>
<td>8</td>
</tr>
<tr>
<td>≥ 2 cm and &lt; 5 cm</td>
<td>39</td>
<td>-91.22</td>
<td>29</td>
</tr>
<tr>
<td>≥ 5 cm</td>
<td>11</td>
<td>-94.24</td>
<td>19</td>
</tr>
</tbody>
</table>

**Abbreviations:** GM, geometric mean; GMR, geometric mean ratio

**Conclusions:** Abemaciclib + ANZ is an effective treatment with manageable toxicities in pts with HR+/HER2- early BC. Abemaciclib-driven change in Ki67 was not associated with disease stage, baseline LN involvement, tumor grade, or tumor size.
Title: ER+ breast cancers resistant to prolonged neoadjuvant letrozole exhibit an E2F4 transcriptional program sensitive to CDK4/6 inhibitors

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1Vanderbilt University Medical Center, Nashville, TN; 2Dana-Farber Cancer Institute, Boston, MA; 3Instituto Valenciano de Oncologia, Valencia, Spain; 4INCLIVA Biomedical Research Institute, Hospital Clinico Universitario, Valencia, Spain; 5Hospital Arnau de Vilanova, Valencia, Spain and 6Université Paris-Saclay, Gustave Roussy Cancer Campus, Villejuif, France.

Body: Background: Neoadjuvant trials with antiestrogen therapy offer an opportunity to discover functional genomic alterations associated with drug resistance that could, in turn, inform the choice of adjuvant therapy. A high preoperative endocrine prognostic index (PEPI) score after 3-4 months of neoadjuvant therapy with an aromatase inhibitor (AI) correlates with an increased risk of cancer recurrence. This suggests that the residual cancer in the breast after prolonged estrogen deprivation can be considered a surrogate of drug-resistant micro-metastases that harbors molecular alterations causally associated with endocrine resistance.

Results: We performed targeted DNA and RNA-sequencing from paraffin-embedded formalin-fixed tumor sections from a cohort of 68 elderly patients (median 77 years) with stage II-III ER+ breast cancer (BC) treated with letrozole for a median of 7.2 months (range 5.4-9.2) before surgery. With a median follow-up of 58 months (range, 50-80), 13 patients (8 with PEPI $\geq$4, 5 with PEPI 1-3) exhibited a BC recurrence (12 metastatic, 1 loco-regional). The 5-year recurrence free survival was 100%, 85% and 61% for PEPI 0, PEPI 1-3 and PEPI $\geq$4, respectively (p=0.001). Patients with PEPI $\geq$4 continued to exhibit a poor prognosis after adjusting for adjuvant chemotherapy (risk of relapse, HR: 2.84, p=0.052). Resistant tumors (BC relapse and/or PEPI $\geq$4) were enriched in signatures related to cell proliferation, stemness, DNA repair, EGFR and PI3K signaling. Integration of the 47 most upregulated genes (log FC>1; FDR<0.03) in letrozole-resistant tumors with transcription binding data from ChIP-Seq studies showed highly significant overlap with 20 E2F4-regulated genes (p=2.56E-15). These genes were overexpressed in MCF7 and T47D cells adapted to long term estrogen deprivation (LTED) and were markedly suppressed by treatment with the CDK4/6 inhibitor palbociclib but only modestly by fulvestrant or paclitaxel. In patients treated with palbociclib for 14 days before surgery [in the Pre-Operative Palbociclib (POP) trial (NCT02008734)], treatment with the CDK4/6 inhibitor significantly decreased expression of 24 of the 47 (FDR<0.01) most upregulated genes in letrozole-resistant tumors (above); among these were 18 of the 20 E2F4 target genes. We next generated an E2F4 activation signature using the 24 genes enriched in the original cohort of letrozole-resistant tumors that were also downregulated by palbociclib in tumors in the POP trial. This signature was strongly associated with resistance to AIs in the ACOSOG Z1031B neoadjuvant trial: On treatment Ki67<2.7% rate was 18% vs 50% for high and low baseline E2F4 score, respectively (p<0.001). Finally, a high E2F4 signature score was associated with an increased risk of relapse (HR: 2.96, 95% CI:2.18-3.67) and death (HR:1.59, 95% CI:1.32-1.94) in ER+ tumors in the METABRIC cohort.

Conclusions: We have identified a CDK4/6 inhibitor-sensitive E2F4 gene expression signature that is associated with estrogen-independent proliferation in ER+ breast cancers resistant to aromatase inhibitors. This signature can potentially identify patients with ER+ breast cancer who are candidates for adjuvant therapy with CDK4/6 inhibitors in combination with antiestrogens.
Title: TransNEOS: Validation of the Oncotype DX recurrence score (RS) testing core needle biopsy samples from NEOS as predictor of clinical response to neoadjuvant endocrine therapy for postmenopausal estrogen receptor positive (ER+), HER2 negative (HER2-) breast cancer patients

Yutaka Yamamoto¹, Hiroji Iwata², Norikazu Masuda³, Tomomi Fujisawa⁴, Tatsuya Toyama⁵, Masahiro Kashiwaba⁶, Shoichiro Ohtani⁷, Naruto Taira⁸, Takehiko Sakai⁹, Yoshie Hasegawa¹⁰, Rikiya Nakamura¹¹, Hiromitsu Akabane¹², Yukiko Shibahara¹³, Hiroshi Sasano¹⁴, Takuhiryo Yamaguchi¹⁵, Kentaro Sakamaki¹⁶, Calvin Chao¹⁷, Debbie McCullough¹⁷, Naoko Sugiyama¹⁸, and Yasuo Ohashi¹⁹. ¹Kumamoto University, Kumamoto, Japan; ²Aichi Cancer Center Hospital, Nagoya, Japan; ³NHO Osaka National Hospital, Osaka, Japan; ⁴Gunma Prefectural Cancer Center, Maebashi, Japan; ⁵Nagoya City University Graduate School of Medical Science, Nagoya, Japan; ⁶Breastopia Miyazaki Hospital, Miyazaki, Japan; ⁷Hiroshima City Hiroshima Citizens Hospital, Hiroshima, Japan; ⁸Okayama University Hospital, Okayama, Japan; ⁹Cancer Institute Hospital of Japanese Foundation for Cancer Research, Tokyo, Japan; ¹⁰Hiroshima Municipal Hospital, Hiroshima, Japan; ¹¹Chiba Cancer Center, Chiba, Japan; ¹²Hokkaido P.W.F.A.C. Asahikawa-Kosei General Hospital, Asahikawa, Japan; ¹³Tohoku University Graduate School of Medicine, Sendai, Japan; ¹⁴Graduate School of Medicine, The University of Tokyo, Tokyo, Japan; ¹⁵Genomic Health, Inc. and ¹⁶Chuo University, Tokyo, Japan.

Body: Background: Neoadjuvant therapy for locally advanced breast cancer has the potential to improve surgical therapeutic outcomes without sacrificing the survival advantages of adjuvant therapy. However, determining whether ER+ patients (pts) will respond to neoadjuvant (NA) chemotherapy (CT) or hormone therapy (HT) can be difficult. Not all ER+ pts respond to NACT, while response to NAHT can vary across ER+ pts. Thus, the ability to select pts more likely to benefit from NAHT would represent progress in clinical management of breast cancer. NEOS is a randomized phase III study assessing long-term prognosis of ER+ primary breast cancer with/without adjuvant CT following NAHT (UMIN 000001090, http://www.umin.ac.jp/). We used archived core biopsy tumor samples from the NEOS study to validate the RS result as a predictor of clinical response and its association with successful breast conserving surgery (BCS) in pts treated with 6 months of NAHT.

Methods: NEOS enrolled 904 postmenopausal pts with ER+, HER2-, clinically node negative (cN0) breast cancer to evaluate whether adjuvant CT was necessary for pts who responded to NAHT. In this current study, we enrolled pts with tumors ≥2cm from the NEOS study. Biopsy samples of 333 pts were assessed for the Oncotype DX assay. Response to NAHT was recorded as complete/partial response (CR/PR), or stable/progressive disease (SD/PD).

Primary endpoint of this study was to evaluate clinical response (CR/PR) between pts with low (<18) and high (≥31) RS result. Secondary endpoints include evaluating the relationships between clinical response and continuous RS results, and other covariates including age, tumor size, grade, Ki67 by IHC, ER and PR single gene scores, and ER and proliferation gene group scores by RT-PCR.

Results: The analysis included 294 pts with median age of 63 yrs, median tumor size of 25mm, and 66% were nuclear grade 1. 156 (53.0%), 83 (28.6%) and 54 (18.4%) cases were low, intermediate, and high RS groups by Oncotype DX, respectively. Six (2%), 126 (42.8%), 149 (50.3%), 13 (4.4%) cases experienced CR, PR, SD, PD as clinical response, respectively, similar to that of all NEOS pts. Clinical response rate was 54%, 42% and 22% in low, intermediate, and high RS groups, respectively. The proportion of pts with clinical response was significantly higher in the low RS group vs the high RS group (p<0.001). In univariate analyses, continuous RS was significantly associated with clinical response (p<0.001), along with ER (p=0.02), PR (p<0.001), and ER gene group score (p<0.001). Other covariates were not associated with clinical response.

Conclusion: The Oncotype DX RS test in core biopsy samples is validated as a predictive assay for clinical response of NAHT in postmenopausal, ER+/HER2-, cN0, primary early breast cancer pts. Further results on the association of RS results with BCS outcomes following NAHT will be presented. These results when combined with previously published data on RS in NACT studies help guide pts with ER+, HER2- breast cancer with NAHT vs NACT treatment options to maximize clinical response.
**Title:** Ki67 changes and PEPI score in the LORELEI trial: A phase II randomized, double-blind study of neoadjuvant letrozole plus taselisib versus letrozole plus placebo in postmenopausal women with ER-positive/HER2-negative early-stage breast cancer

**Methods:** 334 postmenopausal women with newly diagnosed ER+/HER2-, untreated, Stage I-III operable breast cancer and evaluable tumor tissue for PIK3CA genotyping were randomized (1:1) to receive daily letrozole (2.5 mg) with either taselisib (4 mg on a 5 days on/2 days off schedule) or placebo for 16 weeks, followed by surgery. Tumor tissue collection was performed at baseline, week 3 (W3) and at surgery. Secondary objectives included, but were not restricted to, ORR assessed by MRI in patients with PIK3CA wild type (WT) tumors, ORR using alternative methods of tumor assessment (ultrasound, mammogram and clinical breast exam) in all patients and patients with PIK3CA mutant and WT tumors, central assessment of Ki67 at different timepoints (baseline, W3 and surgery), and the centrally derived PEPI score. Central Ki67 was assessed by two independent readers blinded to treatment arms and PIK3CA status (Vall D’Hebron Institute of Oncology, Barcelona).

**Results:** ORR by centrally assessed MRI was similar in the two treatment arms in patients with PIK3CA WT tumors (45.7 vs 40.4% for taselisib and placebo, respectively). ORR assessed by breast US was also significantly higher with taselisib compared to placebo in all randomized patients and in the PIK3CA mutant cohort. The highest concordance rate between MRI and other imaging modalities was found with breast ultrasound (53.7%). Centrally assessed Ki67 changes are reported in Table 1. Ki67 values decreased from baseline to W3 and from baseline to surgery in both treatment arms. No significant differences in the decrease of Ki67 values between treatment arms were detected. Unplanned analysis of Complete Cell Cycle Arrest (CCCA) at W3 was numerically higher with taselisib than with placebo in all randomized patients (49.6% vs 38.5%) and in the PIK3CA mutant cohort (60.9% vs 47.5%). Due to the variability in timing between the last dose of taselisib (median time 11 days; interquartile range 6-16 days) and tissue collection at surgery, considering the half-life of taselisib of approximately 40 hours, centrally derived PEPI score is not interpretable.

<table>
<thead>
<tr>
<th>Ki67 proportional changes, %</th>
<th>Taselisib + letrozole</th>
<th>Placebo + letrozole</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline to W3</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>-83.8</td>
<td>-80.4</td>
</tr>
<tr>
<td>PIK3CA mutant</td>
<td>-84.5</td>
<td>-79.1</td>
</tr>
<tr>
<td>PIK3CA WT</td>
<td>-82.8</td>
<td>-81.1</td>
</tr>
<tr>
<td><strong>Baseline to surgery</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Body: Background:** Taselisib is an oral, potent, selective inhibitor of Class I PI3-kinase (PI3K) alpha, gamma, and delta isoforms with enhanced activity against PIK3CA mutant cancer cells. LORELEI trial demonstrated a significant improvement in ORR (objective response rate) centrally assessed by MRI with neoadjuvant taselisib plus letrozole compared to letrozole plus placebo in all randomized patients as well as in the PIK3CA mutant cohort (Saura et al, ESMO 2017).

Body: Background: Taselisib is an oral, potent, selective inhibitor of Class I PI3-kinase (PI3K) alpha, gamma, and delta isoforms with enhanced activity against PIK3CA mutant cancer cells. LORELEI trial demonstrated a significant improvement in ORR (objective response rate) centrally assessed by MRI with neoadjuvant taselisib plus letrozole compared to letrozole plus placebo in all randomized patients as well as in the PIK3CA mutant cohort (Saura et al, ESMO 2017).
Conclusion: Among the investigated alternative methods for assessing ORR, breast ultrasound performed similar to MRI. Decrease in the Ki67 values from baseline to W3 and to surgery were observed in both treatment arms. The time interval between taselisib cessation and tissue collection at surgery are being further investigated.
Clinical trial information: NCT02273973
Title: Genomic instability and poor antiproliferative response to aromatase inhibitor treatment: A POETIC study

Eugene F Schuster¹, Pascal Gellert¹, Corrinne V Segal¹, Elena López-Knowles¹, Richard Buus¹, James Morden², John Robertson³, Judith Bliss³, Ian Smith⁴, Mitch Dowsett¹,⁴ and POETIC Trial Management Group and Trialists². ¹Breast Cancer Now Research Centre at The Institute of Cancer Research, London, United Kingdom; ²Clinical Trials and Statistics Unit at The Institute of Cancer Research, Sutton, United Kingdom; ³University of Nottingham, Nottingham, United Kingdom and ⁴Ralph Lauren Centre for Breast Cancer Research, Royal Marsden Hospital, London, United Kingdom.

Body: Background:
More than 20% of early-stage patients with estrogen positive (ER+) disease relapse. Higher levels of the proliferation marker Ki67, and lack of reduction of Ki67 in response to AI indicate poorer prognosis. Somatic mutations have been the focus of research in treatment resistance. However, recurrent somatic copy number alterations (SCNAs) are more common and affect more genes in primary breast cancer (BC) than somatic mutations. Previous studies have suggested an increased risk of recurrence for patients with high genomic instability and for patients with loss of heterozygosity (LOH) at the TP53 locus, but it is unknown if these SCNA events impact response to AI treatment. In addition, LOH and mutations at the TP53 locus had a higher risk of recurrence than LOH or mutations at TP53 alone. We hypothesised that genomic instability and SCNAs at particular loci would be increased in early BC patients with high baseline Ki67, and particularly in patients with high Ki67 despite pre-operative AI therapy.

Methods:
In a substudy of POETIC (UK-wide, phase III, randomised trial with 4483 women testing perioperative AI in postmenopausal women with early BC), SNParray technology was used to determine SCNAs in baseline and surgical tumour core-cuts and blood from 76 patients (59 AI-treated, 17 controls). Proliferation rate was estimated as percentage (%) of cancer cells staining for Ki67 by IHC. Poor AI responders (PR, <60% reduction in Ki67 between baseline and surgery, n=31) and good AI responders (GR, >75% reduction in Ki67, n=28) were selected from POETIC samples. Mutation data from exome sequencing was available for tumours from 75 of the patients.

Results:
The fraction of the genome with SCNAs correlated with Ki67 expression in both baseline and surgical samples (baseline Spearman $r_{ho}=0.5$, $p < 10^{-5}$; surgical Spearman $r_{ho}=0.44$, $p < 10^{-5}$). In paired baseline vs surgical samples, 24% of samples showed discordance in SCNAs that covered > 10% of the genome. The samples showing the highest discordance were from PRs.
The fraction of the genome with LOH was greater in PR (median PR 20%, GR 10%, $p = .065$), and the best SCNA to predict the fraction of the genome altered in a sample were segments with LOH at Chr17p13.3 (adjusted $p < .001$, logistic regression). There was a higher percentage of patients with LOH at Chr17p13.3 that contains the TP53 gene in the PR compared to GR group (PR 71%, GR 39%, $p = .029$), and integration of previously generated mutation data with SCNA showed that 9 out of 31 PRs have mutations and LOH at the TP53 locus compared to 3 out of 28 GRs ($p = 0.16$).

Conclusions:
There is discordance between the observed SCNAs in paired samples with high genomic instability and multiple biopsies may be needed to confidently assess all SCNAs. However, LOH at Chr17p13.3 is a biomarker for genomic instability and frequency of LOH is significantly greater in patients that show a poor response to AI treatment. Finally, high genomic instability is associated with high proliferation rates at baseline and surgery after 2 weeks of AI treatment suggesting de novo resistance in tumours with high instability that may lead to a higher rate of recurrence seen in these patients.
Title: Adjuvant palbociclib plus endocrine therapy for hormone receptor positive/HER2 negative breast cancer: A phase II feasibility study

Erica L Mayer¹, Angela M DeMichele², Hao Guo¹, Kathy D Miller³, Hope S Rugo¹, Bryan Schneider³, Adrienne G Waks¹, Steven E Come⁵, Theresa Mulvey⁶, Cynthia Huang Bartlett⁷, Maria Koehler⁷, William Barry¹, Eric P Winer¹ and Harold J Burstein¹.

¹Dana-Farber Cancer Institute, Boston, MA; ²University of Pennsylvania Abramson Cancer Center, Philadelphia, PA; ³Indiana University Melvin and Bren Simon Cancer Center, Indianapolis, IA; ⁴University of California San Francisco Helen Diller Family Comprehensive Cancer Center, San Francisco, CA; ⁵Beth Israel Deaconess Medical Center, Boston, MA; ⁶Massachusetts General Hospital Cancer Center, Boston, MA and ⁷Pfizer Inc, New York, NY.

Body: Background:
The CDK4/6 inhibitor palbociclib (P) combined with endocrine therapy (ET) prolongs progression-free survival in previously untreated and treated hormone receptor positive/HER2 negative (HR+/HER2-) metastatic breast cancer (MBC). The most common toxicity with P is neutropenia, typically non-cumulative and uncomplicated. Given observed benefits of P in metastatic BC, this single arm phase II trial was designed to determine the feasibility and toxicity of combination adjuvant P and ET for HR+/HER2- early BC (EBC).

Methods:
Eligible patients (pts) had HR+/HER2- stage II (not T2N0)-III EBC, with prior completion of 3-24 mo of ET (either AI or tamoxifen) without significant adverse events (AE). Pts received P at 125 mg daily, 3 wk on/1 wk off in a 28d cycle, plus continuous ET, for planned duration 2 yrs. Pts were removed from study for toxicity, non-adherence, or other events related to tolerability; pts who recurred or completed 2 yrs of therapy were censored for the primary endpoint. The primary objective was to evaluate the treatment discontinuation rate at 2 yrs; a rate of ≥50%, would indicated a non-feasible treatment duration (null hypothesis). Discontinuation rates at 2 yrs are estimated by Kaplan Meier with 95% confidence bands. A sample size of 160 pts provided 92% power to reject the null hypothesis using a one-sided alpha = 0.025 if the true rate of discontinuation is ≤33.3%, and accounting for a censoring rate of up to 20% over the 2 yrs. Secondary endpoints include toxicity, adherence, QOL, and pharmacogenomics.

Results:
Between 3/2014 and 11/2015, 162 pts initiated P; the majority had stage III EBC (52%) and received prior chemotherapy (63%). As of 05/2017, 120 (74%) have completed at least 1 yr of P + ET, and 50 (31%) have completed 2 yrs of P + ET. Early discontinuation of protocol treatment was reported for 59 pts (36%), including 49 events (30%) related to protocol-mandated (9%) and non-mandated (21%) tolerability. The cumulative rate of all discontinuations was 15.1% at 6 mos, 20.9% at 12 mos and 27.8% at 18 mos. Half of all non-mandated discontinuations occurred within the first 6 mos of initiation of therapy, and the rate decreased with greater provider and pt education. Median duration of pts still on treatment is 20 mos (inter-quartile range: 18 to 21 mos). The rate of grade 3/4 neutropenia was 77%, with 0 cases of febrile neutropenia. Other common all-grade P-related AE > 20% included fatigue 65%, alopecia 25%, mucositis 24%, and anemia 24%. 32% of pts required one dose reduction, 16% required two. There have been 2 BC recurrence events and 1 chemotherapy-related AML. Updated data for the primary analysis of feasibility and tolerability, as well as pharmacogenomics, QOL, and adherence, will be presented.

Conclusions:
In this single arm phase II trial, the majority of pts have completed at least 1 year of adjuvant P + ET therapy, with no new toxicity signals. Non-protocol discontinuations have decreased with education. Updated results for the primary analysis will be presented. As in the MBC setting, extended duration palbociclib appears feasible and tolerable for most pts. The efficacy of 2 years of P and ET will be addressed by the phase III PALLAS trial (NCT NCT02513394).
2017 San Antonio Breast Cancer Symposium

Publication Number: PD5-07

Title: A phase III randomized trial of anastrozole and fulvestrant versus anastrozole or sequential anastrozole and fulvestrant as first-line therapy for postmenopausal women with metastatic breast cancer: Final survival outcomes of SWOG S0226

Rita S Mehta1, William E Barlow2, Kathy S Albain3, Ted A Vandenbarg4, Shakher R Dakhil5, Nagendra L Tirumali6, Danika L Lew2, Daniel F Hayes7, Julie R Gralow8, Hannah M Linden9, Robert B Livingston10 and Gabriel N Hortobagyi11. 1UCIMC, Orange, CA; 2SWOG Statistical Center, Seattle, WA; 3Loyola University Chicago Stritch School of Medicine, Maywood, IL; 4London Health Sciences Center, London, ON, Canada; 5Wichita Community Clinical Oncology, Wichita, KS; 6Northwest CCOP/Northwest, Portland, OR; 7University of Michigan, Ann Arbor, MI; 8Puget Sound Cancer Consortium, Seattle, WA; 9University of Washingtons, Seattle, WA; 10University of Arizona/Arizona Cancer, Tuscon, AZ and 11MD Anderson, Houston, TX.

Body: Background: Anastrozole depletes estrogen via aromatase inhibition and fulvestrant binds and degrades estrogen receptor. In a Phase III trial we compared the concurrent use of these agents to anastrozole alone or sequential anastrozole and fulvestrant in first-line therapy of hormone receptor-positive metastatic breast cancer in postmenopausal women, and demonstrated improved progression-free (PFS) and overall survival (OS)-NEJM 2012. Now we report PFS and OS five years after the initial positive findings. Methods: A total of 707 patients were randomized to either 1 mg anastrozole P.O. daily (Arm 1) or to the combination of anastrozole and fulvestrant (Arm 2). Fulvestrant was administered as a loading dose of 500 mg on day 1, 250 mg on days 14, 28 and monthly thereafter. Randomization was stratified by adjuvant tamoxifen use. The primary endpoint was PFS with OS a secondary outcome. 40% patients not in visceral crisis crossed over to fulvestrant after progression on arm 1. Analysis of survival was by 2-sided stratified log-rank tests and Cox regression using intent-to-treat. Subset analyses include treatment effect by adjuvant tamoxifen exposure, initial sites of metastases and time from diagnosis. Results: There were 646 PFS events (328 and 318 for arms 1 and 2, respectively) among 694 eligible patients (345 and 349, respectively). Overall, median PFS was 13.5 months for arm 1 and 15.0 months for the arm 2 (log-rank p=0.007; HR=0.81 (95% CI 0.69-0.94)). This benefit extended similarly in visceral and non-visceral subgroups. In subset analysis for Arms 1 and 2, respectively, in tamoxifen-naive women (60%, n=414), median PFS was 12.7 vs. 16.7 months (log-rank p=0.002; HR=0.73 (95% Cl 0.60-0.89) while in women exposed to tamoxifen, median PFS was 13.9 vs. 13.6 months (log-rank p=0.57; HR=0.93 (95% Cl 0.73-1.19)). An improved OS in the combination arm was seen, median OS 42 and 50 months in arms 1 and 2, based on 261 and 247 deaths, respectively (log-rank p=0.028; HR=0.82 (95% Cl 0.69-0.98)). In subset analysis in tamoxifen-naive women, median OS was 40.3 vs. 52.2 months for Arms 1 and 2, respectively (log-rank p=0.007; HR=0.73 (95% Cl 0.58-0.92)) while in women exposed to tamoxifen, median OS was 43.5 vs. 48.2 months (log-rank p=0.85; HR=0.97 (95% Cl 0.74-1.27). Patients with initial diagnosis >10 years benefitted most from the combination (HR=0.66 (95% Cl 0.49-0.89)) regardless of tamoxifen exposure. Patients in Arm 1 who crossed over had post-progression survival similar to post-progression survival of Arm 2 patients. Conclusion: The addition of fulvestrant to anastrozole was associated with improved long-term PFS and OS compared to anastrozole alone, despite the use of fulvestrant at a dose lower than the approved, and despite the substantial cross over to fulvestrant after progression on anastrozole alone. The benefit was especially notable in those without recent exposure to adjuvant endocrine therapy. Ongoing translational medicine studies will further refine the need for up front fulvestrant. ClinicalTrials.gov:NCT00075764. Funding: NIH/NCI U10CA180888, U10CA180819 and AstraZeneca.
2017 San Antonio Breast Cancer Symposium

Publication Number: PD5-08

Title: Elacestrant, oral selective estrogen receptor degrader (SERD) in patients with ER positive (ER+)/HER2- advanced breast cancer: Updated phase 1 efficacy, safety and pharmacodynamic results

Aditya Bardia1, Peter Kabos2, Sharon Wilks3, Donald Richards4, Wael Harb5, Richard Elledge6, Dannie Wang7, Hai Jiang7, Fiona Garner7, Alison O'Neill7 and Virginia Kaklamani6. 1Massachusets General Hospital, Harvard Medical School, Boston, MA; 2University of Colorado, Aurora, CO; 3Cancer Care Centers of South Texas, San Antonio, TX; 4Texas Oncology, Tyler, TX; 5Horizon Oncology Center, Lafayette, IN; 6CTRC, University of Texas Health Science Center San Antonio, San Antonio, TX and 7Radius Health Inc, Waltham, MA.

Body: Background: The treatment of advanced ER+ breast cancer remains a clinical challenge with the majority of patients eventually progressing due to acquisition of resistance to endocrine therapy. Elacestrant is a novel, nonsteroidal, oral SERD that has demonstrated dose-dependent degradation of ER, and down-regulation of ER-regulated genes in preclinical studies. In multiple in vivo patient-derived xenograft models of breast cancer, including those harboring ESR1 mutations, elacestrant demonstrated significant single agent antitumor activity. In a separate biomarker study RAD1901-106, robust FES-PET suppression was seen following a 400 mg daily dose in patients with metastatic breast cancer (DeVries et al SABCS 2016).

Methods: In a phase-1 Study RAD1901-005 (ClinicalTrials.gov ID: NCT02338349), postmenopausal women with advanced ER+ breast cancer who had received prior endocrine therapy (no limit on number of prior endocrine therapies) were enrolled in dose escalation cohorts based on a standard 3+3 design, followed by a safety expansion cohort at 400 mg qd, the recommended phase-2 dose (RP2D). ESR1 mutation status was determined from circulating tumor DNA (ctDNA) samples, and clinical outcomes were evaluated based on standard RECIST v1.1 criteria.

Results: As of April 28, 2017, a total of 47 patients were enrolled in the RAD1901-005 trial, with the majority (n= 40) at the 400 mg qd RP2D. Patients were heavily pre-treated (median lines of prior endocrine therapy = 3), including prior fulvestrant, mTOR inhibitor and/or CDK4/6 inhibitor. As of April 28, 2017, in patients treated at the RP2D with RECIST measurable disease, the objective response rate (ORR) was 23% (5/22), median duration of response was 17.4 weeks, and clinical benefit rate (CBR) at 24 weeks was 42% in patients with mature datasets. ESR1 mutations were detected in 50% of patients at baseline (n= 20) with a CBR at 24 weeks of 50%; of the 11 patients with ESR1 mutations with RECIST measurable disease, 4 had confirmed partial responses (ORR = 31%). Elacestrant was generally well-tolerated, with the most common adverse events being nausea/vomiting (grade 1/2 = 50%), dyspepsia (grade 1/2 = 23%). RAD1901 exposure was dose dependent, with a t1/2 of approximately 30-40 hours. Serial analysis of ctDNA demonstrated decreases in the allelic fraction of ESR1 mutations in response to treatment.

Conclusion: Elacestrant has demonstrated compelling evidence of single agent clinical activity, with confirmed partial responses in patients with ESR1 mutations and heavily pre-treated patients with multiple lines of therapy including fulvestrant, mTOR and/or CDK 4/6 inhibitor, warranting further development in ER+ breast cancer. Updated clinical outcomes, including ORR progression-free survival, and biomarker data will be presented at the meeting.
Title: Fulvestrant for hormone receptor-positive advanced breast cancer in patients with visceral vs non-visceral metastases: Findings from FALCON, FIRST, and CONFIRM

John FR Roberston¹, Angelo Di Leo², Mehdi Fazal³, Jasmine Lichfield⁴ and Matthew J Ellis⁵. ¹University of Nottingham, Royal Derby Hospital Centre, Derby, United Kingdom; ²Hospital of Prato, Prato, Italy; ³AstraZeneca, Gaithersburg, MD; ⁴AstraZeneca, Cambridge, United Kingdom and ⁵Lester and Sue Smith Breast Center, Baylor Clinic, Baylor College of Medicine, Houston, TX.

Body: BACKGROUND
Patients with hormone receptor-positive (HR+) locally advanced or metastatic breast cancer (LA/MBC) and non-visceral metastases (non-VM) generally have a better prognosis than patients with visceral metastases (VM). However, in the absence of visceral crisis, endocrine therapy (ET) remains an effective treatment option in both patient groups. This descriptive analysis examined the treatment effect of fulvestrant 500 mg vs comparators in postmenopausal patients with HR+ LA/MBC, with or without VM.

METHODS
Three randomized studies of fulvestrant 500 mg for postmenopausal HR+ LA/MBC were included. The Phase 3 FALCON study (NCT01602380) compared fulvestrant 500 mg with anastrozole in patients without any prior ET (n=462; fulvestrant 500 mg: 58.7% with VM; anastrozole: 51.3% with VM). The Phase 2 FIRST study (NCT00274469) compared fulvestrant 500 mg with anastrozole in patients who had not received ET for advanced disease (n=205; fulvestrant 500 mg: 47.1% with VM; anastrozole: 56.3% with VM). The Phase 3 CONFIRM study (NCT00099437) compared fulvestrant 500 mg with fulvestrant 250 mg (n=736; fulvestrant 500 mg: 56.6% with VM; fulvestrant 250 mg: 52.9% with VM); patients had received prior ET for adjuvant/advanced disease. The treatment effect of fulvestrant 500 mg vs comparator ET was determined using log-rank tests.

RESULTS
In FALCON, there was a greater treatment effect with fulvestrant 500 mg vs anastrozole for progression-free survival (PFS) in the non-VM group (hazard ratio [HR] 0.59) vs the VM group (HR 0.99). A consistent treatment effect was observed for fulvestrant 500 mg vs comparator for PFS in FIRST (non-VM HR 0.58; VM HR 0.82) and CONFIRM (non-VM HR 0.72; VM HR 0.86). Median PFS of fulvestrant 500 mg vs comparator in non-VM and VM subgroups was: 22.3 months (m) vs 13.8 m and 13.8 m vs 15.9 m, respectively, in FALCON; 34.0 m vs 21.3 m and 9.8 m vs 9.9 m in FIRST; and 10.4 m vs 5.9 m and 4.7 m vs 4.0 m in CONFIRM. Clinical benefit rate with fulvestrant 500 mg vs anastrozole in FALCON was 87.4% vs 75.2% in the non-VM group, and 71.9% vs 73.1% in the VM group.

Overall survival (OS) in FALCON (31% maturity) showed a greater treatment effect with fulvestrant 500 mg vs anastrozole in the non-VM group vs the VM group (HR 0.60 vs 1.09). In terms of OS, in FIRST there was a greater treatment effect with fulvestrant 500 mg vs anastrozole in the non-VM group compared with the VM group (HR 0.68 vs 0.86). In CONFIRM, improved OS was observed with fulvestrant 500 mg vs fulvestrant 250 mg; this treatment effect was consistent in non-VM (HR 0.78) and VM subgroups (HR 0.83).

CONCLUSIONS
In three studies, an improved treatment effect of fulvestrant 500 mg vs comparator ET for HR+ LA/MBC was observed in patients with non-VM. The treatment effect of fulvestrant 500 mg vs comparator for PFS across all three studies appeared consistent. A reduced treatment effect of fulvestrant 500 mg vs comparator was generally seen in patients with VM, although fulvestrant 500 mg was still as effective as, or slightly more effective than, the comparator. These data suggest that patients without VM may benefit more from fulvestrant 500 mg than patients with VM.
2017 San Antonio Breast Cancer Symposium

**Publication Number:** PD5-10

**Title:** A first-in-human phase I study to evaluate the oral selective estrogen receptor degrader (SERD), GDC-0927, in postmenopausal women with estrogen receptor positive (ER+) HER2-negative metastatic breast cancer (BC)

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**Body: Background:** Modulation of estrogen activity and/or synthesis is the mainstay therapeutic strategy in the treatment of ER+ BC. However, despite the effectiveness of available endocrine therapies, many patients ultimately relapse or develop resistance to these agents via estrogen-dependent and estrogen-independent mechanisms, including mutations in ESR1 affecting the ER ligand binding domain that drive ER-dependent transcription and proliferation in the absence of estrogen. ER antagonists that are efficacious against ligand-dependent and ligand-independent, constitutively active ESR1 mutant tumors may be of substantial therapeutic benefit. GDC-0927 (formerly known as SRN-927) is a novel, potent, non-steroidal, orally bioavailable, selective ER antagonist/ER degrader (SERD) that induces tumor regression in ER+ BC patient-derived xenograft models.

**Methods:** A phase I dose escalation study with 3+3 design was conducted in postmenopausal women with ER+ (HER2-) metastatic BC (progressing ≥ 6 months on endocrine therapy and with ≤ 2 prior chemotherapies in the advanced or metastatic setting) to determine the safety, pharmacokinetics (PK) and the recommended Phase 2 dose (RP2D) of GDC-0927. Pharmacodynamic (PD) activity was assessed with [18F]-fluoroestradiol (FES)-PET scans. Plasma PK samples (after single dose and at steady state), CT scans, and when feasible, pre and on-study tumor biopsies were obtained

**Results:** From March 16, 2015 to March 17, 2017 patients (pts) with a median age of 53 years (range 44-69) and a median number of prior therapies for MBC 4 (range 1-7) were enrolled at 3 total daily dose levels (600, 1000, 1400 mg) once daily (QD) given orally with fasting (n = 12). Increases in GDC-0927 exposure were approximately dose proportional. Treatment related adverse events (AEs) were all grade 1 or 2. The most common treatment-related AEs were nausea (54%, n = 7), diarrhea (46%, n = 6), elevated aspartate aminotransferase (39%, n = 5) and anemia, constipation, (each 31%, n = 4). Treatment interruption was required for 2 pts due to nausea and vomiting. Of those pts with FES-PET avid disease at baseline (9 of 12), all post-therapy scans showed complete or near complete (> 90%) suppression of FES uptake to background levels, including pts with ESR1 mutations. Evidence of reduced ER levels and Ki67 staining was observed in on-treatment biopsies. Five of 12 pts (1 at 600 mg and 4 at 1400 mg) were on study ≥ 24 weeks (CBR = 41.6 %) with the best overall response of stable disease with 1 patient (ESR1 mt+ D538G) on study for over 490 days. There were no dose limiting toxicities and no SAEs related to study drug. R2PD was 1400 mg and was selected for single arm dose-expansion which is now complete with last patient enrolled on March 17, 2017. Updated results from dose-escalation and dose-expansion will be presented at the meeting (N = 43).

**Conclusions:** GDC-0927 appears well-tolerated to date with PK exposure supporting QD dosing, evidence of robust PD target engagement, and encouraging anti-tumor activity in heavily pretreated pts with advanced or metastatic ER+ BC, including pts with ESR1 mutations.
Title: Ribociclib in combination with everolimus and exemestane in men and postmenopausal women with HR+/HER2− advanced breast cancer following progression on a CDK4/6 inhibitor: Efficacy and updated safety and pharmacokinetic results from phase 1 of the TRINITI-1 study

Aditya Bardia1, Denise A Yardley2, Sara Hurvitz3, Gail Wright4, Rebecca Moroose5, Cynthia Ma6, Lowell Hart7, Elizabeth Tan-Chiu8, Sibal Blau9, Tara Santit10, Robert Dichmann11, Amelia Zelnak12, Angela DeMichele13, Amy Clark13, Tania Small14, Chris Tucci14, Tanay S Samant14, Das Purkayastha14, Megan Karuturi15 and Stacy Moulder15. 1Massachusetts General Hospital Cancer Center, Boston, MA; 2Sarah Cannon Research Institute, Nashville, TN; 3University of California, Los Angeles Medical Center, Santa Monica, CA; 4Florida Cancer Specialists, New Port Richey, FL; 5UF Health Cancer Center - Orlando Health, Orlando, FL; 6Washington University School of Medicine, St. Louis, MO; 7Florida Cancer Specialists and Research Institute, Fort Myers, FL; 8Florida Cancer Care, Plantation, FL; 9Northwest Medical Specialties, Puyallup, WA; 10Yale School of Medicine Smilow Cancer Hospital, New Haven, CT; 11Central Coast Medical Oncology Corporation, Santa Maria, CA; 12Atlanta Cancer Care (Northside Hospital), Cumming, GA; 13University of Pennsylvania Abramson Cancer Center, Philadelphia, PA; 14Novartis Pharmaceuticals Corporation, East Hanover, NJ and 15The University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Background: Endocrine therapy in combination with cyclin-dependent kinases 4 and 6 (CDK4/6) inhibitors significantly improves progression-free survival compared with endocrine monotherapy in patients with hormone receptor–positive (HR+), human epidermal growth factor receptor 2–negative (HER2−) advanced breast cancer (ABC). However, the optimal regimen after progression on CDK4/6 inhibitors is unclear. The known interaction between CDK4/6 and the phosphatidylinositol 3-kinase/mammalian target of rapamycin pathway as well as the improved antitumor activity with the addition of ribociclib (R) to everolimus (EVE) in preclinical studies suggest a potential for this triplet combination in ABC to restore endocrine sensitivity in HR+, HER2− disease that has progressed on CDK4/6 inhibitors. TRINITI-1 is a Phase 1/2 open-label study of R + EVE + exemestane (EXE). Preliminary Phase 1 safety, tolerability, and pharmacokinetics (PK) of the TRINITI-1 study have been reported previously. We now report Phase 1 efficacy and updated PK and safety.

Methods: In the Phase 1 dose escalation stage, 2 doses of continuous R daily dosing (250 mg/d or 300 mg/d) were evaluated; doses of EVE and EXE were fixed. Men and postmenopausal women with HR+, HER2− ABC who had progressed on 1 to 3 lines of systemic endocrine therapy, had measurable disease and/or lytic/mixed bone lesions, and had an Eastern Cooperative Oncology Group performance status score of ≤1 were eligible (ClinicalTrials.gov identifier, NCT02732119). Progression on a CDK4/6 inhibitor was allowed, but was only required for patients in Phase 2. Patients with visceral crisis, unstable central nervous system metastases, progression after treatment with >1 CDK4/6 inhibitor, or clinically significant heart disease were excluded. The primary objective of Phase 1 was identification of the maximum tolerated dose and/or the recommended Phase 2 dose of R + EVE + EXE. Secondary objectives included preliminary antitumor activity, safety, tolerability, and PK analyses.

Results: Seventeen evaluable patients were enrolled in Phase 1 between June 14 and December 12, 2016. Twelve patients (70.6%) received prior CDK4/6 inhibitor therapy for ABC. As of July 31, 2017, the clinical benefit rate at week 24 per local assessment was 58.8% in all patients (50% in CDK4/6 inhibitor-refractory patients and 80% in CDK4/6 inhibitor-naive patients), with a best response of confirmed partial response in 1 CDK4/6 inhibitor-naive patient on 250 mg/d ribociclib. Eleven patients (64.7%) discontinued triplet therapy, most commonly because of progressive disease (41.2%). One patient on 300 mg/d ribociclib had a dose-limiting toxicity of Grade 4 febrile neutropenia. Preliminary PK data found exposure of 2.5 mg/d EVE with 250 or 300 mg/d ribociclib and 25 mg/d EXE to be within the therapeutic range for 5-10 mg/d EVE monotherapy and exposure of continuous ribociclib to be consistent with single-agent exposure. Updated PK data will be presented. The most common Grade 3/4 adverse events (>15%) were neutropenia (59%), anemia (18%), and pneumonitis (18%). Febrile neutropenia occurred in 1 patient in each cohort. No increase in liver function tests or corrected QT (Frederica's formula) >480 ms (any grade) occurred.
Body: Background: BELLE-2 (NCT01610284) met its primary endpoint of a statistically significant improvement in progression-free survival (PFS) with the pan-phosphoinositide 3-kinase (PI3K) inhibitor BUP+FUL compared with PBO+FUL in postmenopausal pts with HR+, HER2– ABC (Baselga et al. Lancet Oncol 2017). Here, we report the final results for OS.

Methods: Postmenopausal women with HR+, HER2– ABC refractory to aromatase inhibitors were enrolled in this randomized, double-blind, placebo-controlled, Phase III study. Pts were randomized (1:1) to receive either oral BUP (100 mg/day) or PBO plus intramuscular FUL (500 mg per standard of care). Randomization was stratified by PI3K pathway status in archival tumor tissue (activated \(\text{PIK3CA} \) mutation or loss of phosphatase and tensin homolog expression), non-activated or unknown) and visceral disease status (present or absent). Baseline \(\text{PIK3CA} \) mutation status in circulating tumor (ct) DNA was assessed in a subset of pts. The key secondary endpoint was OS, defined as time from randomization to date of death due to any cause, in the full population (PI3K pathway activated, non-activated and unknown), main study cohort (PI3K pathway activated and non-activated) and PI3K pathway-activated subpopulation. Other secondary endpoints included safety. OS by \(\text{PIK3CA} \) status in ctDNA was also investigated.

Results: 1147 pts received BUP+FUL or PBO+FUL. In the full population and main cohort, OS results trended in favor of BUP+FUL vs PBO+FUL, but were not statistically significant (Table). Post-treatment use of antineoplastic medications was balanced between both arms. In pts with PI3K pathway-activated status, OS improved by approximately 6 months (Table). In pts with mutant \(\text{PIK3CA} \) ctDNA status, there was a slight trend in favor of BUP+FUL vs PBO+FUL (Table).

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<th>Median OS, months</th>
<th>HR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full population N=1147</td>
<td>BUP+FUL n=576</td>
<td>33.2</td>
<td>0.87 (0.74–1.02)</td>
</tr>
<tr>
<td></td>
<td>PBO+FUL n=571</td>
<td>30.4</td>
<td></td>
</tr>
<tr>
<td>Main cohort n=851</td>
<td>BUP+FUL n=427</td>
<td>30.9</td>
<td>0.91 (0.75–1.09)</td>
</tr>
<tr>
<td></td>
<td>PBO+FUL n=424</td>
<td>28.9</td>
<td></td>
</tr>
<tr>
<td>PI3K pathway activated n=372</td>
<td>BUP+FUL n=188</td>
<td>33.6</td>
<td>0.81 (0.61–1.08)</td>
</tr>
<tr>
<td></td>
<td>PBO+FUL n=184</td>
<td>27.5</td>
<td></td>
</tr>
<tr>
<td>ctDNA (\text{PIK3CA} ) mutant n=200</td>
<td>BUP+FUL n=87</td>
<td>26.0</td>
<td>0.81 (0.56–1.17)</td>
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</table>
The safety profile of BUP+FUL was consistent with that previously reported, with no new safety concerns. Grade 3/4 adverse events with at least 5% difference between BUP+FUL vs PBO+FUL, respectively, were increased alanine aminotransferase (25.7% vs 1.1%), increased aspartate aminotransferase (18.0% vs 2.8%), hyperglycemia (15.4% vs 0.2%) and rash (8.0% vs 0.0%).

Conclusions: The BELLE-2 OS results showed a trend in favor of BUP+FUL, but did not meet statistical significance. More selective PI3K inhibition may improve treatment benefit and safety compared with pan-PI3K inhibition. Further assessment of the predictive benefit of ctDNA-confirmed *PIK3CA* mutations in this setting is required.
Prevalence of PDL1 and tumor infiltrating lymphocytes (TILs) in primary and metastatic TNBC

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**Background:** Metastatic Triple Negative Breast Cancer (TNBC) is an aggressive breast cancer subtype with a high unmet medical need and limited therapeutic options. PDL1 on immune cells (IC) and TILs were associated with activity to the anti-PDL1 mAB atezolizumab in mTNBC (Schmid AACR 2017). PDL1 IC >=1% is the diagnostic hypothesis for TNBC atezolizumab studies. The goal of the current project was to evaluate the prevalence of PDL1 and TILs in primary and metastatic TNBC samples.

**Methods:** FFPE tumor tissue from pathology documented TNBC tissues were centrally tested for PDL1 IHC on tumor cells (TC) and infiltrating immune cells (IC) (VENTANA SP142 assay) and quantified as percentage of tumor area. TILs were evaluated as a percentage of tumor area and PD-L1 gene (CD274) expression was quantified by Fluidigm.

**Results:** 669 tumors were evaluable for PD-L1 by IHC and TILs. 280 samples were annotated as primary tumors and 179 as metastases with 210 denoted as unknown. PDL1 IC and TC immunostaining was correlated to PD-L1 transcript expression \((r = 0.55 \text{ and } r = 0.51, \text{ respectively})\). 84% of samples had no tumor cells expressing PDL1 and 16% had at least 1% of PDL1 in TC. 44% of the samples had no PDL1 in the immune cells, 35% expressed PDL1 IC \(\geq1\% \text{ to } <5\%\) and 21% PDL1 IC \(\geq5\%). PDL1 distribution was similar between primary and metastases, with median of 1% and 0% for PDL1 IC and TC, respectively. Analyses of anatomical locations suggest that PDL1 IC is highest in lymph nodes (median IC = 3%), lowest in liver metastases (median IC = 0.5%) while breast tissue is intermediate (median IC = 1%). The correlation between PDL1 IC and TILs is \(r = 0.69\). TILs were numerically higher in primary compared to metastases (10% vs 5%). Anatomical TIL distribution confirmed liver metastases as least infiltrated, while breast, lymph node and lung metastases with similarly higher values (5% vs 10%, respectively). Concordance analyses in intra-individual synchronous and asynchronous matched pairs were performed at a cutoff of PDL1 IC \(\geq1\%). Concordance of matched specimens was 82% in 11 synchronous pairs and 75% in 20 asynchronous pairs.

**Conclusions:** Prevalence of PDL1 IHC is similar between primary and metastatic TNBC samples. Although PDL1 IC and TILs are highly correlated, TILs are less prevalent in metastases compared to primary tissue. Anatomical location may influence prevalence of both PDL1 IC and TILs. Concordance of PDL1 in matched pairs is high, suggesting PDL1 IC may be a stable biomarker.
Immuneological differences between primary and metastatic breast cancer

Borbala Szekely¹,², Veerle Bossuyt³, Xiaotong Li¹, Marina Bainè³, Andrea Silber¹, Tara Sanft¹, Erin Hofstatter¹, Sarah Mougalian¹, Saneea Baghwagar¹, Veronique Neumeister³, Vasiliki Pelekanou³, Christos Hatzis¹ and Lajos Pusztai¹.¹ Yale Cancer Center, Yale School of Medicine, New Haven, CT; ²National Institute of Oncology, Budapest, Hungary and ³Yale School of Medicine, New Haven, CT.

**Background:** Little is known about how the immune microenvironment of breast cancer evolves during disease progression. Immunological differences between primary and metastatic lesions may explain discordant results of clinical trials that showed low tumor response rates with immune checkpoint therapy in metastatic breast cancer but high rates of pathologic complete response in early stage disease. The goal of this project was to examine TIL counts, PD-L1 protein expression and immune gene mRNA expression in primary tumors (P) and metastatic lesions (M).

**Methods:** FFPE blocks of primary breast cancers and core needle biopsies of matching distant metastases from 54 patients (n=104 samples). TIL count was assessed on H&E slides for 39 paired tissues and is reported as % of TIL in the stroma. PD-L1 protein expression was detected with immunohistochemistry (IHC, E1L3N antibody) in 36 pairs, samples with > 1% cells showing staining were considered PD-L1 positive which was determined separately for tumor and stroma. The expression of 770 immune-related genes was measured using the Nanostring PanCancer Immune Gene Panel in 31 P and 17 M, including 10 paired cases. Genes were organized into 14 immune cell type (total T, Th1, Treg, Total CD8, exhausted CD8, Cytotoxic T, B, NK, NK-CD56, Mast cell, CD45, Dendritic cell, macrophage, neutrophil) and 22 immune function metagenes. Differences in mean expression in P and M were assessed using Fisher exact and Mann-Whitney tests without adjustment for multiple comparisons due to overlap in metagene membership.

**Results:** Mean TIL counts (14% vs 20%, p=0.026) and stromal PD-L1 positivity by IHC (14% vs 54%, p=0.004) were significantly lower in M. PD-L1 positivity in tumor cells was similar (25% in M vs 42%, in P p=0.14). The total TIL gene expression score (2.48 vs 2.8 p=0.018) and all immune cell type metagenes, except neutrophils, had lower absolute expression levels in M. The relative abundance of neutrophils (0.035 vs -0.38, p=0.0001) and macrophages (0.62 vs 0.38 p=0.0013) increased in M. Among the 22 immune function metagenes, T, B and NK cell functions, cytotoxicity, chemokine and TNF superfamily expression, regulation and pathogen defense were significantly lower in M and none showed significantly increased expression in M. Pro-inflammatory/immune-activating cytokines of IL-6, CCL-5,-12,-19,-22 and CXCL-5,-9,-10,-11 were all significantly lower in M. The greatest drop was seen for CXCL-9 (2243 vs 422, p<0.0001) and CCL-19 (2537 vs 309, p<0.0001). No cytokine showed increased expression in M. Only 6 immune genes (C7, GPI, MAPK1, TAB1, TLR5, PVR) showed significantly higher (p<0.05) expression in M, the greatest increase was for Complement C7 (member of the terminal complement pathway membrane attack complex; 484 vs 3499, p=0.03) and GPI (glucose 6-phosphate isomerase that induces immunoglobulin secretion; 1967 vs 3011, p=0.01).

**Conclusions:** Breast cancer metastases exist in an attenuated immune microenvironment. Most immune cell subtypes, immune functions, and immune-associated gene expression are lower in M compared to P, consistent with immune escape. Metastatic lesions have higher relative abundance of macrophages and neutrophils, which suggest new therapeutic opportunities.
**2017 San Antonio Breast Cancer Symposium**

**Publication Number:** PD6-03

**Title:** Distribution of microsatellite instability, tumor mutational load, and PD-L1 status in molecularly profiled invasive breast cancer

Elias Obeid¹, Angela Ellerbrock², Elizabeth Handorf¹, Lori Goldstein¹, Zoran Gatalica², David Arguello², Sandra M Swain³, Claudine Isaacs³, Jeff Vacirca¹, Antoinette Tan⁵ and Lee Schwartzberg⁶. ¹Fox Chase Cancer Center, Philadelphia, PA; ²Caris Life Sciences, Phoenix, AZ; ³Lombardi Comprehensive Cancer Center, Washington DC; ⁴New York Cancer & Blood Specialists, East Setauket, NY; ⁵Levine Cancer Institute, Carolinas HealthCare System, Charlotte, NC and ⁶West Cancer Center, University of Tennessee, Memphis, TN.

**Body:**

**Background:** Recent data indicate a promising response to immune check point blockade (ICB) in patients with breast cancer. Pembrolizumab, a humanized monoclonal antibody against programmed death 1 (PD-1) receptor and one of several ICB agents in development, was given an FDA approval for all MSI (microsatellite instability)-high solid tumors. MSI incidence in breast cancer is not fully elucidated. Other biomarkers being explored in possible relationship to ICB activity include PD-1 ligand (PD-L1) status and tumor mutational load (TML). In this study, we aimed to explore the incidence of these biomarkers in invasive breast cancers.

**Methods:** A retrospective data analysis of patients profiled by commercial next-generation sequencing (NGS) at Caris Life Sciences was performed. MSI results were either high, stable, or equivocal. MSI was calculated by comparing repeat-insertions or deletions across over 7,000 microsatellite sequences in the patient sample to the hg19 reference genome. Samples with repeat variances in more than 45 microsatellites were classified as MSI-High PD-L1 expression was evaluated using immunohistochemical analysis (IHC), with clone SP-142 (Roche Diagnostics). A sample was considered positive if there was >5% membranous staining of tumor cells. Tumor mutational load was calculated as a total number of non-synonymous somatic mutations identified per megabase of the genome coding area with high being greater than or equal to 17.

**Results:** A total of 9,627 breast cancer cases were queried from the Caris Life Sciences database. The mean age (±SD) was 56.8 ± 12.4 years (range 20-90). The tumor distribution was 60.7% hormone receptor (HR) positive (ER and/or PR) and HER2 negative, 9.5% HER2 positive (with HR negative or positive), and 29.8% triple negative (negative for ER, PR and HER2). Of all cases, there were 5,203 tested for PD-L1 status, 354 (6.8%, 95% CI 6.2-7.5%) were positive. Of 1,440 tumors tested for MSI status, 15 (1.04%, 95% CI 0.58-1.71%) were either high (8) or equivocal (7), the rest were MSI-low. Tumor mutational load (TML) was available on 1,766 tumors, of which 55 (3.1%, 95% CI 2.4-4.0%) were high. Seven out of the 8 MSI-high tumors were also TML-high. Four out of the 8 MSI-high breast cancers were triple negative.

**Conclusion:** In this large dataset of molecularly profiled breast cancer, MSI was observed in about 1% of the breast tumors tested. Overall, modest positivity of TML, PD-L1, and MSI of all invasive breast cancers was observed. The percentage of patients that had at least one of these biomarkers that may confer responsiveness to ICB is planned and will be further stratified by subtype. MSI-high breast cancers mostly overlapped with those that were TML-high. Future research is needed to show the clinical utility of these biomarkers in response to ICB. Updated data will be presented.
Title: HDAC inhibitors modulate immune checkpoint blockade in breast cancer

Manuela T Barberio¹, Scott Thomas¹, Niwa Ali¹, Jeenah Park¹, Michael D Rosenblum¹, Alfredo Budillon², Nela Pawlowska¹ and Pamela N Munster¹. ¹University of San Francisco, San Francisco, CA and ²Experimental Pharmacology Unit - National Cancer Institute, Pascale, Naples, Italy.

Body: Background: Breast cancer is one of the most common diseases, second only to lung cancer as the leading cause of cancer death in women. In particular hormone sensitive metastatic breast cancer (MBC) and triple-negative breast cancer (TNBC) remains a therapeutic challenge as resistance develops in almost all patients. Despite aggressive treatment strategies, survival is poor due to the development of resistance, prompting the need for novel approaches. Here, we characterize the molecular mechanisms underlying epigenetic modifiers and in particular histone deacetylase inhibitors (HDACi) as priming modulators of immunotherapy, with a specific focus on TNBC and hormone resistant breast cancers. HDACi represent a new class of anticancer agents that can reverse hormone therapy resistance, resulting in prolonged anti-tumor responses in patients. In addition to their effects on estrogen receptor (ER) signaling, HDACi can also influence immune cell function, including but not limited to modulation of Foxp3+ regulatory T-cells (Tregs), tumor-infiltrating lymphocyte composition, as well as induction of the co-inhibitory receptors PD-L1 and PD-1. Under normal conditions the PD-1/PD-L1 pathway down-regulates cytotoxic T-cell activity to maintain immune homeostasis. Cancer cells exploit this pathway in the tumor microenvironment to suppress cytotoxic T-cell activation, significantly diminishing the anti-tumor immune response.

In breast cancer, PD-L1 expression is less frequent and mainly found in TNBC, HER2+, ER- and PR- tumors. Increased PD-L1 expression correlates with increased tumor infiltrated lymphocytes (TILs) and these criteria together are indicative of improved response rates in breast cancer patients.

The PD-1/PD-L1 pathway represents one of the primary immunosuppressive drivers in multiple types of cancer. Thus, inhibiting PD-1/PD-L1 interactions may prevent T-cell suppression and reactivate immunosurveillance mechanisms necessary for tumor cell eradication.

Methods & Results: Evaluation of basal PD-L1 expression in a range of human and mouse breast cancer cell lines by western blotting and real-time PCR identified TNBC and HER2+ cells as the highest expressing cells. Testing different epigenetic modifiers, we found that HDACi were able to up-regulate PD-L1 mRNA and protein in a time-dependent manner up to 72 hours. This was a direct transcriptional effect induced by HDACi and was confirmed even in tamoxifen resistant breast cancer cells, characterized by increased basal expression of PD-L1 as compared to the parental cells.

To define the role of epigenetic priming in promoting immune cell activation, we co-cultured tumor cells and human peripheral blood mononuclear cell (PBMCs) and performed comprehensive immunophenotyping by flow cytometry. HDACi were able to up-regulate PD-L1 on tumor cells independent of PBMCs, while exhibiting a selective decrease in the frequency of immunosuppressive Tregs.

Conclusion: Our data demonstrate that the combination of HDACi with immune checkpoint inhibitors represents a novel therapeutic anti-tumor strategy and warrants further clinical evaluation for the treatment of TNBC and hormone resistant breast cancer.
2017 San Antonio Breast Cancer Symposium

Publication Number: PD6-05

Title: Distinct tumor microenvironments stratify triple negative breast cancer into immune subtypes

Tina Gruosso¹, Mathieu Gigoux¹, Nicholas Bertos¹, Venkata SK Manem², Dongmei Zuo¹, Sadiq MI Saleg¹, MargaritaSouleimanova¹, Hong Zhao¹, Radia M Johnson¹, Anne Monette³, Valentina Muñoz Ramos¹, Michael T Hallett¹, John Stagg³, Réjean Lapointe³, Atilla Omeroglu⁴, Sarkis Meterissian⁴, Laurence Buisseret⁶, Gert Van den Eyden⁵, Roberto Salgado⁵, Marie-Christine Guiot⁴, Benjamin Haibe-Kains² and Morag Park¹. ¹Goodman Cancer Research Center, McGill University, Montreal, Canada; ²Princess Margaret Cancer Centre, University of Toronto, Toronto, Canada; ³Centre de Recherche du Centre Hospitalier de l'Université de Montréal et Institut du Cancer de Montréal, Montreal, Canada; ⁴McGill University Health Centre and McGill University, Montreal, Canada and ⁵Breast Cancer Translational Research Laboratory, Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium.

Body: Background:
Triple negative breast cancer (TNBC) are especially difficult to treat effectively. While only 20-30% of TNBC patients respond to chemotherapy in the neoadjuvant setting, overall outcome remains poor for non-responding patients. Engaging the immune system promises optimal personalized cancer therapy as mounting evidence suggests that immune-checkpoint inhibitor immunotherapies may become a therapeutic option for TNBC patients. The presence of CD8+ T cells, a crucial component of the cytotoxic arm of the adaptive immune response, is associated with good clinical outcome in TNBC patients. Specifically, it is the efficient CD8+ T cell invasion and infiltration in the tumor that is associated with good outcome. On the other hand, some tumors accumulate CD8+ T cells in the tumor-associated stroma with poor infiltration in the tumor epithelium. These patients show poor outcome. As CD8+ T cell infiltration in the tumor is a crucial step to mount an efficient anti-tumor response, we thus wondered how the tumor microenvironment affects CD8+ T cell invasion into the tumor epithelial compartment of the TNBC tumors.

Methods:
To identify potential stroma-dependent mechanisms that potentiate or inhibit CD8+ T cells invasion into the tumor epithelium, we coupled analysis of spatial patterns of CD8+ T cell localization by Immunohistochemistry (IHC) and performed gene expression profiling of laser-capture microdissected tumor-associated stroma (as well as matched epithelium and bulk tumor) from 38 TNBC chemotherapy-naive primary cases. GSEA-based Metasignatures were derived from bulk tumor gene expression data from our cohort. To investigate the compartment of origin of the pathways identified via the Metasignatures, the (LCM)-derived tumor stromal and epithelial gene expression were analyzed.

Results:
CD8+ T cell quantification in different compartments of the tumor identify 3 main subgroups of TNBC based on CD8+ T cell localization. Importantly we developed a 2-step classification scheme based on CD8+ T cell localization. We developed metasignatures following our 2 steps classification and identified key bulk tumor metasignatures that showed prognostic value in an independent cohort. In addition the matched LCM gene expression from the tumor epithelium and stromal compartments allowed us to identify the compartment of origin.

Importantly, while 1 group of TNBC tumor was showing a significant anti-tumor response, the 2 other groups showed absence of such environment. The 2 non inflamed immune subtypes showed distinct phenotypes and biologies associated with poor anti-tumor response that we validated by immunohistochemistry and fluorescence. These results highlight different potential mechanisms that lead to immune evasion and allow us to stratify TNBC into immune subgroups.
Understanding the complexity of macrophage and mesenchymal stem cell interactions to improve treatment outcome for IBC patients

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Cells within the tumor microenvironment, including but not limited to macrophages and mesenchymal stem cells (MSCs), can promote the phenotype and aggressiveness of inflammatory breast cancer (IBC). For example, co-injection of MSCs with SUM149 IBC cells significantly increased the clinical features of IBC such as skin invasion and metastasis. Our preliminary work showed that MSCs can be educated by co-culture with M1 polarized (anti-tumor) or M2 polarized (pro-tumor) mouse Raw macrophages. Such education of MSCs by M2 Raw macrophages leads to increased IL6 secretion by MSCs, relative to M1-educated or uneducated MSCs. M2-educated MSCs also have increased migration toward IBC cell lines SUM149 and IBC3, effects that can be blocked by an anti-IL6 antibody. Co-culture with M2-educated MSCs also enhances migration and mammosphere formation of IBC cells. Radiation response of IBC cells upon interactions with cells from the tumor microenvironment was also analyzed. Preliminary work shows that co-culture of IBC cells (SUM149 and KPL4) with M2-polarized human THP1 macrophages, prior to ionizing radiation, mediates radiation resistance of IBC cells, and this effect can be decreased by either adding HDL lipoproteins during co-culture period or by STAT6 inhibitors that block IL4/IL13-mediated phosphorylation of STAT6 and M2-polarization in THP1 macrophages. MSCs can also be polarized into either a MSC1 phenotype or a MSC2 phenotype by exposure to toll-like receptor (TLR) ligands TLR4 or TLR3, respectively. Indoleamine-pyrrole 2,3-dioxygenase (IDO) expression in MSCs is a marker of MSC2 polarization that is induced after exposure with TLR3 ligand (PolyIC) relative to MSC1 (TLR4 stimulated; LPS-treated) or parental MSCs. Similar to macrophage polarization, while MSC1 mediates anti-tumor effects, MSC2 are immunosuppressive and thus contribute to tumor growth. Preliminary work also shows that co-culture of IBC cells with MSC2 mediates radioresistance and this can be decreased as well by exposure to HDL during co-culture period prior to radiation. HDL protective effects, in part, can be explained by decreased expression of TLR3-induced IDO mRNA levels in MSC2. In the present work, we extended the above mentioned observations regarding the crosstalk between mouse Raw macrophages and MSCs by analyzing the effect of co-culture of human THP1 macrophages (parental designated as M0, M1- or M2-polarized) with MSCs on the IDO mRNA expression in MSCs, a marker of MSC2 polarization. Surprisingly, co-culture of M1-polarized THP1 with MSCs resulted in a robust increased expression of IDO mRNA in MSC relative to parental MSC (uneducated) or MSCs co-cultured with M2-THP1. Further studies are needed to determine the effects of increased IDO expression in MSC, upon M1-THP1 co-culture, on the aggressive behavior of IBC cells and whether this could be altered with IDO inhibitors. Our results suggest that there could be inter-species differences between mouse and human macrophages on the education of human MSCs. Based on our findings we propose testing a combination of STAT6 inhibitors that reverse M2-polarization of macrophages and IDO inhibitors that can decrease MSC2 phenotype mediated by TLR3 exposure and/or M1-THP1 education.
Clinical significance of CD73 expression in triple-negative breast cancer from the BIG 02-98 adjuvant phase III clinical trial

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Background: CD73 is an ecto-enzyme that promotes tumor immune escape through the production of immunosuppressive extracellular adenosine in the tumor microenvironment. Several CD73 inhibitors and adenosine receptor antagonists are being evaluated in phase I clinical trials.

Objective: To investigate the prognosis significance of CD73 in human triple-negative breast cancer.

Design and setting: This is a prospective-retrospective biomarker analysis. Using multiplex immunofluorescence and image analysis, we assessed CD73 protein expression on tumor cells, tumor-infiltrating leukocytes and stromal cells on full-face sections from formalin-fixed paraffin-embedded primary breast tumors.

Participants: 122 samples of triple-negative breast cancer from the BIG 02-98 adjuvant phase III clinical trial were included in our analysis. This trial compared the addition of taxanes to anthracyclines-based chemotherapy in node-positive breast cancer.

Results: Our results demonstrated that high levels of CD73 expression on epithelial tumor cells were significantly associated with reduced disease-free survival (DFS) and overall survival (OS) in patients with triple-negative breast cancer. Using the median as a threshold between low and high levels of CD73 on epithelial cells, hazard ratios (HR) adjusted for grade, number of positive lymph nodes and tumor size, were of 2.21 (95% confidence interval (CI): 1.15-4.25); p=0.02 for DFS and of 2.47 (95%CI: 1.21-5.07); p=0.01 for OS. CD73 expression negatively correlated with tumor immune infiltration (Spearman's R= -0.50, p<0.0001). Patients with high levels of CD73 and low levels of tumor-infiltrating leukocytes had the worse clinical outcome (HR: 4.24 (1.90-9.45), p<0.001 for DFS, HR: 3.91 (1.65-9.31), p=0.002 for OS) compared to patients with low CD73 and high tumor-immune infiltration. Flow cytometric analysis of tumor-infiltrating leukocytes revealed a high frequency of CD73-expressing B cells and higher CD73 expression on tumor-infiltrating myeloid cells and natural killer cells compared to peripheral blood.

Conclusion and relevance: Taken together, our study provides further support that CD73 expression is associated with a poor prognosis and reduced anti-tumor immunity in human triple-negative breast cancer and that targeting CD73 could be a promising strategy to reprogram the tumor microenvironment in this breast cancer subtype.
Title: Analysis of immune infiltrates (assessed via multiplex fluorescence immunohistochemistry) and immune gene expression signatures as predictors of response to the checkpoint inhibitor pembrolizumab in the neoadjuvant I-SPY 2 trial

Michael Campbell¹, Christina Yau¹, Alexander Borowsky², Scott Vandenberg¹, Denise Wolf¹, David Rimm³, Rita Nanda⁴, Minetta Liu⁵, Lamorna Brown-Swigart¹, Gillian Hirst¹, Smita Asare¹, Laura van’t Veer², Doug Yee⁶, Angie DeMichele⁷, Don Berry⁸ and Laura Esserman¹. ¹University of California, San Francisco; ²University of California, Davis; ³Yale University; ⁴University of Chicago; ⁵Mayo Clinic Cancer Center; ⁶University of Minnesota; ⁷University of Pennsylvania and ⁸University of Texas.

Body: Background: Pembrolizumab (Pembro), an anti-PD-1 immune checkpoint inhibitor, has been approved for the treatment of a variety of cancers including melanoma, non-small cell lung cancer, head and neck squamous cell carcinoma, and urothelial carcinoma. Pembro was recently evaluated in HER2- breast cancer patients in the neoadjuvant I-SPY 2 TRIAL and graduated in the triple negative (TN), HR+HER2-, and HER2- signatures. HER2- patients were randomized to receive Pembro+paclitaxel followed by doxorubicin/cyclophosphamide (P+T -> AC) vs. T -> AC. We and others have shown that TN breast cancers tend to have high numbers of immune infiltrates, including T cells and tumor associated macrophages (TAMs). We evaluated expression signatures representing 14 immune cell types (TILs, T cells, CD8 T cells, exhausted T cells, Th1, Tregs, cytotoxic cells, NK, NK CD56dim, dendritic cells, mast cells, B cells, macrophages, and neutrophils) as specific predictors of response to Pembro.

Methods: Data from 248 patients (Pembro: 69; controls: 179) were available. Pre-treatment biopsies were assayed using Agilent gene expression arrays. Signature scores are calculated by averaging cell type specific genes. All I-SPY 2 qualifying biomarker analyses follow a pre-specified analysis plan. We used logistic modeling to assess biomarker performance. A biomarker is considered a specific predictor of Pembro response if it associates with response in the Pembro arm but not the control arm, and if the biomarker x treatment interaction is significant (likelihood ratio test, p<0.05). This analysis is also performed adjusting for HR status as covariates, and within receptor subsets. For successful biomarkers, we use Bayesian modeling to estimate the pCR rates of ‘predicted sensitive’ patients in each arm. Our statistics are descriptive rather than inferential and do not adjust for multiplicities of other biomarkers outside this study.

Results: 10 out of the 14 cell-type signatures tested are associated with response in the Pembro arm. Higher expression levels of 9 of these cell-type signatures are associated with higher pCR rates (T cells, exhausted T cells, Th1, cytotoxic cells, NK, NK CD56dim, dendritic cells, B cells, and macrophages), whereas higher mast cell signature expression is associated with non-pCR. Interestingly, many of these same signatures also associate or trend towards association with response in the control arm; and in a model adjusting for HR status, only 3 of these signatures (Th1, B cells and dendritic cells) show significant interaction with treatment. Within the whole population and the TN subtype, the dendritic cell signature is the strongest predictor of specific response to Pembro (OR/1SD: 4.04 and 4.4, LR p < 0.001 overall and in TN). Although other immune signatures (T cells, exhausted T cells, NK, and macrophages) also associate with response in the Pembro arm in the TN subtype, only the dendritic cell and Th1 signatures have a significant interaction with treatment. In contrast, in the HR+HER2- subtype, only 3 signatures (Th1, B cells, and mast cells) associate with response to Pembro; but none of these signatures have significant interaction with treatment. Of note, in both the Pembro and control arms, HR+HER2- patients with higher average mast cell marker expression have lower pCR rates (OR/1SD: 0.33 and 0.51, LRp: 0.006 and 0.04 in Pembro and control arm).

Conclusion: As expected, multiple immune cell expression signatures are predictive of response in the Pembro arm; but only dendritic cells and Th1 cells are specific to Pembro in both the population as a whole and the TN subtype. Interestingly, the presence of mast cells may impede response, especially in HR+HER2- patients. Correlation of these signatures with multiplex-IF immune markers is pending.
The immune microenvironment in hormone receptor-positive breast cancer patients and relationship to treatment outcome following preoperative chemotherapy plus bevacizumab

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Body: Background: Hormone receptor-positive (HR+) tumors have fewer tumor-infiltrating lymphocytes (TILs) and lower response rates to immune checkpoint inhibitors (ICI), either as single agents or in combination with chemotherapy, than triple negative cancers. However, some HR+ cancers do respond to ICI and biomarkers that accurately reflect the immune microenvironment may help guide the use of ICI therapy. Prior evidence suggests that macrophage-related immune pathways may be relevant to the pathophysiology of HR+ BC.

Methods: HR+/HER2- patients were identified from a prospective trial of preoperative bevacizumab (preop bev) followed by bev with adriamycin/cyclophosphamide/paclitaxel dose-dense chemotherapy (chemo). Tumor samples were collected at diagnosis and surgery (pre-tx and post-tx), and PD-L1 expression (by immunohistochemistry), TILs, and Nanostring PanCancer Immune Profiling Panel were evaluated on both pre-tx and post-tx specimens. Pre-tx whole transcriptome sequencing was performed. Pathologic response at surgery was centrally assessed by Miller-Payne (MP) and residual cancer burden (RCB) scores. An immune score was calculated for each pre-tx specimen by integrating 10 published immune signatures. Immune cell subsets were inferred from bulk transcriptional data using CIBERSORT and immune cell-specific signatures from MSigDB.

Results: 55 patients who received trial therapy and had at least 1 evaluable specimen were included for analysis. Pre-tx TILs and tumor PD-L1 (tPD-L1) scores are shown in the table. 18% of pre-tx tumors had “high” (≥10%) TILs and “high” TILs were associated with significantly higher immune signature score (p=0.004). Immune score correlated highly with proportion of CIBERSORT anti-tumor M1 macrophages as well as CD8 T-cell signatures (r>0.65 and p<0.001). Higher pre-tx TILs, tPD-L1, or immune score were each significantly associated with more favorable RCB and MP in unadjusted analyses (all Spearman p<0.01 for pathologic markers; ANOVA p<0.04 for immune score). After adjustment for age and tumor grade, higher pre-tx TILs and tPD-L1 were associated with favorable RCB (p<0.01 for both), and higher pre-tx tPD-L1 correlated with favorable MP (p=0.03). Pathologic complete response occurred in 4 pts; all 4 had high pre-tx TILs, pre-tx tPD-L1, or both. Among patients with residual disease, large changes (>5%) in TILs or tPD-L1 from pre-tx to post-tx were rare: 2 pts each had large changes in TIL or tPD-L1 score (N=38/N=31 pairs, respectively).

Conclusions: High levels of tumor-lymphocyte interaction were seen in only a minority of untreated HR+ breast tumors, and did not typically change with chemo plus bev. An immune score derived from bulk RNAseq correlated with histological observations in these specimens. Nonetheless, TILs, tPD-L1, and signature-derived immune score were significantly associated with pathologic response to preop treatment in HR+ disease. Early data suggest that the role of M1 macrophages in HR+ tumors warrants further investigation.

<table>
<thead>
<tr>
<th>Score</th>
<th>TILs (N=50 evaluable)</th>
<th>Tumor PD-L1 (N=51)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>0 pts (0%)</td>
<td>28 pts (55%)</td>
</tr>
<tr>
<td>&gt;0-5% (low)</td>
<td>19 (38%)</td>
<td>18 (35%)</td>
</tr>
<tr>
<td>≥5-10% (intermediate)</td>
<td>22 (44%)</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>≥10% (high)</td>
<td>9 (18%)</td>
<td>2 (4%)</td>
</tr>
</tbody>
</table>
Body: Background: Patients with previously untreated mTNBC typically receive cytotoxic chemotherapy as first-line therapy for metastatic disease. However, efficacy and safety are suboptimal. KEYNOTE-086 (NCT02447003) is a multicohort, single-arm, phase 2 study of pembrolizumab monotherapy for mTNBC. In cohort B, we assessed the safety and antitumor activity of pembrolizumab as first-line therapy for patients with PD-L1-positive mTNBC.

Methods: Key eligibility criteria for cohort B were age ≥18 y, centrally confirmed TNBC, no prior systemic anticancer therapy for metastatic disease, ECOG performance status 0-1, measurable disease per RECIST v1.1 by central review, no radiographic evidence of brain metastases, and a tumor PD-L1 combined positive score (CPS) ≥1. Pembrolizumab 200 mg was given once every 3 wk for 24 mo or until disease progression, intolerable toxicity, or investigator or patient decision. Tumor imaging was performed every 9 wk for 12 mo and every 12 wk thereafter. Clinically stable patients with radiologic progression could remain on pembrolizumab until progression was confirmed on subsequent assessment. Primary end point was safety. Secondary end points included ORR, duration of response, and PFS (all RECIST v1.1 by central review) and OS.

Results: Of the 206 patients with tumors evaluable for PD-L1 expression, 128 (62%) had PD-L1 CPS ≥1. Of these, 84 met all eligibility criteria and enrolled. All patients were women, median age was 52.5 y, 29 (35%) had ECOG PS 1, 40 (48%) had elevated LDH, 55 (65%) had visceral ± nonvisceral metastases, and 73 (87%) received prior (neo)adjuvant therapy. All patients received ≥1 pembrolizumab dose, and after median follow-up of 10.6 mo, 18 (21%) remained on pembrolizumab. Treatment-related AEs occurred in 53 (63%) patients and were of grade 3-4 severity in 7 (8%); no patients died or discontinued pembrolizumab because of treatment-related AEs. The most common treatment-related AEs were fatigue (26%), nausea (13%), and diarrhea (12%). No grade 3-4 treatment-related AE occurred in >1 patient. The most common immune-mediated AE was hypothyroidism (10%). Three patients had complete response and 16 had partial response for an ORR of 23% (95% CI 15-33). Of the 11 patients with a best response of stable disease, 1 had stable disease for ≥24 wk, leading to a disease control rate of 24% (95% CI 16-34). Of 12 responses were ongoing at data cutoff, and median duration of response was 8.4 mo (range 2.1+ to 13.9+). Median PFS was 2.1 mo (95% CI 2.0-2.2), with an estimated 6-mo PFS rate of 26%. Median OS was 16.1 mo (95% CI 11.3- NR), with an estimated 6-mo OS rate of 83%.

Conclusions: Pembrolizumab monotherapy continues to demonstrate a favorable safety profile and robust, durable antitumor activity in patients with PD-L1-positive, previously untreated mTNBC.
Title: An open-label, multitumor, phase II basket study of olaparib and durvalumab (MEDIOLA): Results in germline BRCA-mutated (gBRCAm) HER2-negative metastatic breast cancer (MBC)

Susan M Domchek, Sophie Postel-Vinay, Yung-Jue Bang, Yeonh H Park, Jerome Alexandre, Jean-Pierre Delord, Antoine Italiano, Benoit You, Sara Bastian, Matthew Krebs, Ding Wang, Saiama Waqar, Helen Angell, Maria Learoyd, Shao-Chun Chang, Christopher Gresty, Pia Herbolsheimer, and Bella Kaufman.

Hospital of the University of Pennsylvania, Philadelphia; Institut Gustave Roussy, Villejuif, France; Seoul National University Hospital, Seoul, South Korea; Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea; Cochin Hospital, Paris, France; Institut Claudius Regaud-Oncopole, Toulouse, France; Institut Bergonié, Bordeaux, France; Lyon-Sud University, Lyon, France; Kantonsspital Graubünden, Chur, Switzerland; The Christie NHS Foundation Trust, Manchester, United Kingdom; Henry Ford Hospital, Detroit; Washington University Medical School, St Louis, MO; AstraZeneca, Cambridge, United Kingdom; AstraZeneca, Gaithersburg, MD and Sheba Medical Center, Ramat Gan, Israel.

Body: Background Olaparib is a potent poly(ADP-ribose) polymerase (PARP) inhibitor, with first-in-class approval (400 mg capsules BID) for BRCA-mutated advanced ovarian cancer. Olaparib was recently found to be superior compared with chemotherapy in HER2-negative gBRCAm MBC in the Phase III OlympiAD trial. Olaparib induces DNA damage and genomic instability in gBRCAm tumors, which is hypothesized to result in enhanced immunogenicity. Here, we assess if a combination of olaparib and an anti-programmed cell death ligand-1 (PD-L1) agent, durvalumab, leads to enhanced antitumor activity in one of the four cohorts (NCT02734004).

Methods Individuals with deleterious gBRCA1 or BRCA2 mutations and HER2-negative MBC were eligible. Patients with prior platinum therapy were allowed to enter if they had not experienced disease progression on platinum. No more than two prior lines of chemotherapy were allowed for metastatic disease, and patients with hormone receptor (HR) positive disease had to have at least one line of prior endocrine therapy. Concomitant use of endocrine therapy was not allowed. All patients received olaparib tablets 300 mg PO BID for a 4-week run-in, followed by a combination of olaparib 300 mg PO BID and durvalumab 1.5 g IV q 4 weeks. The combination was continued until progressive disease (PD) by RECIST 1.1 criteria. Tumor assessments were done at baseline, 4 weeks and every 8 weeks thereafter. The primary endpoint was disease control rate (DCR) at 12 weeks, and safety and tolerability of the combination. The secondary endpoints included DCR at 28 weeks, objective response rate, duration of response, progression-free survival (PFS) and overall survival. Biomarker endpoints included PD-L1 expression and evaluation of tumor infiltrating lymphocytes. Olaparib monotherapy has previously demonstrated a median PFS (mPFS) of 6 months in this patient population, and addition of durvalumab was predicted to increase the mPFS to 7.5 months, corresponding to a target DCR of 75% at 12 weeks. This was achievable by enrolling 30 patients using a Bayesian predictive probability design. Results of the first 25 patients are presented.

Results Median age was 46 years (range 29–66); 11 and 14 patients had BRCA1 and BRCA2 mutations, respectively; 13 patients had HR positive disease, and nine had previously received platinum therapy. Median number of prior chemotherapy lines was 1 (range 0-4). Grade 3 or higher adverse events (AEs) were anemia (8%), neutropenia (8%), hemolysis (4%), dyspnea (4%), pancreatitis (4%), fatigue (4%), lymphopenia (4%) and leukopenia (4%). There was one grade 5 AE (dyspnea), which was attributed to disease progression. Observed DCR at 12 weeks was 80%. Eight confirmed responses and five unconfirmed responses were seen. The updated results from primary and secondary endpoints, including DCR at 28 weeks, as well as biomarker and PK data, will be presented.

Conclusions The combination of olaparib and durvalumab was well tolerated, with no apparent overlapping toxicities. The efficacy of the combination at an early time point reached the target DCR in gBRCAm HER2-negative MBC, and warrants further investigation.
Title: Phase 1b/2 study to evaluate eribulin mesylate in combination with pembrolizumab in patients with metastatic triple-negative breast cancer

Sara M Tolaney, Kevin Kalinsky, Virginia Kaklamani, Claudio Savulsky, Martin Olivo, Gursel Aktan, Peter A Kaufman, Dongyuan Xing, Ana Almonte, Soamnauth Misir, Vassiliki Karantza and Sami Diab. 1 Dana-Farber Cancer Institute, Boston, MA; 2 Columbia University Medical Center, New York, NY; 3 University of Texas Health Science Center, San Antonio, TX; 4 Eisai Ltd., Hatfield, United Kingdom; 5 Eisai Inc., Woodcliff Lake, NJ; 6 Merck & Co., Kenilworth, NJ; 7 Norris Cotton Cancer Center, Dartmouth-Hitchcock Medical Center, Lebanon, NH and 8 Rocky Mountain Cancer Centers-Aurora, Aurora, CO.

Body: Background: Eribulin mesylate (ERI), a nontaxane microtubule inhibitor with effects on tumor biology (increased vascular perfusion, reversal of epithelial to mesenchymal transition), is approved as a monotherapy for the treatment of patients (pts) with metastatic breast cancer who received ≥2 prior chemotherapeutic regimens for metastatic disease. In a pooled analysis, ERI significantly prolonged OS compared with capecitabine or treatment of physician's choice in pts with metastatic triple-negative breast cancer (mTNBC; 12.9 vs 8.2 mo, n=428). Pembrolizumab (PEM) is a human programmed death (PD) receptor-1–blocking antibody approved for the treatment of several advanced cancers. In a phase (Ph) 2 study in mTNBC, PEM monotherapy as first-line therapy demonstrated ORR, 23%; median PFS, 2.1 mo [95% CI 2.0-3.9], and in pts pretreated with ≥1 prior chemotherapy demonstrated ORR, 5%; median OS, 8.9 mo [95% CI 7.2-11.2]).

Methods: This open-label Ph 1b/2 trial enrolled pts (aged ≥18 yrs; ECOG PS ≤1) with mTNBC treated with ≤2 prior lines of chemotherapy for metastatic disease. Ph 1b included a safety cohort of ≥6 pts who received intravenous (IV) ERI 1.4 mg/m² on day (d) 1 and d8 and IV PEM 200 mg on d1 of a 21-d cycle. In Ph 2, pts were enrolled based on prior chemotherapy in the metastatic setting [0 vs 1–2 lines]. Primary endpoints: safety, tolerability (Ph 1b), and ORR (Ph 2); secondary endpoints: PFS, OS, and efficacy in PD-L1+ pts.

Results: We report data from 82 of 104 enrolled pts (data cut-off Nov 1, 2016). The RP2D was ERI 1.4 mg/m² on d1 and d8 and PEM 200 mg on d1 of a 21-d cycle. Most common treatment-emergent adverse events (TEAEs) were fatigue (73.2%), nausea (51.2%), peripheral sensory neuropathy (46.3%), alopecia (43.9%), and pyrexia (36.6%). Most common Grade (G) 3 or 4 TEAEs related to ERI: neutropenia (29.3%), peripheral neuropathy (8.5%), and asthenia/fatigue (7.3%). G3/4 immune-related TEAEs related to PEM: 19.5% of pts. TEAEs that led to drug withdrawal/dose reduction: 18.3%/28.0% of pts. G5 events: 3 pts (respiratory failure, pleural effusion, and multiple organ failure; none related to study drug). Response was irrespective of PD-L1 status (Table 1).

<table>
<thead>
<tr>
<th></th>
<th>Overall (n=82)</th>
<th>No prior chemotherapy in metastatic setting (n=48)</th>
<th>1-2 Prior lines of chemotherapy in metastatic setting (n=34)</th>
<th>PD-L1+ (n=35)</th>
<th>PD-L1- (n=36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORR, n (%) [95% CI]</td>
<td>21 (25.6) [16.8, 35.4]</td>
<td>12 (25.0) [14.0, 37.8]</td>
<td>9 (26.5) [13.3, 41.8]</td>
<td>9 (25.7) [12.9, 40.8]</td>
<td>9 (25.0) [12.5, 39.8]</td>
</tr>
<tr>
<td>CBRb, n (%)</td>
<td>25 (30.5)</td>
<td>13 (27.1)</td>
<td>12 (35.3)</td>
<td>10 (28.6)</td>
<td>12 (33.3)</td>
</tr>
<tr>
<td>DCRc, n (%)</td>
<td>46 (56.1)</td>
<td>28 (58.3)</td>
<td>18 (52.9)</td>
<td>19 (54.3)</td>
<td>21 (58.3)</td>
</tr>
<tr>
<td>Median PFS, mo [95% CI]</td>
<td>4.1 [2.3-4.8]</td>
<td>4.1 [2.2-4.9]</td>
<td>3.9 [2.1-6.3]</td>
<td>4.1 [2.1-4.8]</td>
<td>4.1 [2.3-6.3]</td>
</tr>
</tbody>
</table>

Results of the final analysis will be available for presentation.
[95% CI]  

Credible interval from Bayesian analysis;  
clinical benefit rate = CR+PR+SD;  
disease control rate = CR+PR+SD ≥24 weeks.  
NE, not estimable.

**Conclusions:** ERI+PEM was well tolerated and demonstrated activity in pts with mTNBC. The combination resulted in improved ORR, with longer PFS, OS, and comparable TEAEs to those observed with either treatment as monotherapy. Further exploration of this combination is warranted.
Title: Analysis of DNA repair deficiency biomarkers as predictors of response to the PD1 inhibitor pembrolizumab: Results from the neoadjuvant I-SPY 2 trial for stage II-III high-risk breast cancer

Christina Yau1, Denise Wolf1, Lamorna Brown-Swigart1, Gillian Hirst1, Ashish Sanil2, Ruby Singhrao1, I-SPY 2 TRIAL Investigators3, Smita Asare1, Angela DeMichele4, Don Berry2, Laura Esserman1, Laura van’t Veer1, Rita Nanda5, Minetta Liu6 and Douglas Yee7. 1University of California, San Francisco; 2Berry Consultants, LLC; 3QuantumLeap Healthcare Collective; 4University of Pensylvania; 5University of Chicago; 6Mayo Clinic and 7University of Minnesota.

Body: Background: Pembrolizumab (P), an anti-PD-1 immune checkpoint inhibitor, has been approved for treatment of microsatellite instability-high and mismatch repair deficient cancers. In I-SPY 2, patients were randomized to receive standard chemotherapy alone or in combination with an experimental agent. P was one of the experimental agents evaluated in HER2-patients in I-SPY 2 and graduated in the TN, HR+HER2-, and HER2- signatures. We hypothesize that a combination of two signatures predicting response to veliparib/carboplatin therapy in I-SPY 2 [MammaPrint High2 (MP2)/PARPi7-high] and reflecting DNA damage repair deficiency, may also predict response to P. In addition, we also tested 9 gene expression signatures reflecting different aspects of DNA damage and repair: FA, MMR, BER, HR, TLS, NER, NHEJ, DR, and DNA damage sensing (DDS) pathways.

Methods: Data from 249 patients (P: 69 and controls: 180) were available. Pre-treatment biopsies were assayed using Agilent gene expression arrays. All I-SPY 2 qualifying biomarker analyses follow a pre-specified analysis plan. We used logistic modeling to assess biomarker performance. A biomarker is considered a specific predictor of P response if it associates with response in the P arm but not the control arm, and if the biomarker x treatment interaction is significant (likelihood ratio test, p<0.05). This analysis is also performed adjusting for HR status as a covariate, and within receptor subsets, sample size permitting. For successful biomarkers, we use Bayesian modeling to estimate the pCR rates of 'predicted sensitive' patients in each arm. Our statistics are descriptive rather than inferential and do not adjust for multiplicities of other biomarkers outside this study.

Results: MP2 status associates with pCR in P (OR=7.7; p=0.00021), but also to a lesser extent in the control arm (OR=2.4; p=0.045), with an OR ratio of 3.3 which trends toward significance, even after adjusting for HR status (LR p=0.083). A majority of TN patients are MP2; and TN/MP2 patients have an estimated pCR rate of 67% in P (vs. 23% in control). Although only ~30% of HR+/HER2- patients were MP2, their estimated pCR rate in P is 61%, compared to 29% in unselected HR+/HER2- patients. PARPi7 predicted response in the P arm only in the HR+/HER2- group (LR p= 0.025), but not in the population as a whole or the TN subtype. Combining MP2 and PARPi7 into MP2/PARPi7-high did not improve performance over MP2 as a single biomarker. Of the 9 DDR pathway signatures tested, both BER and DDS associate with pCR in P, but only DDS (which includes ATM, ATR, CHEK1-2) associates with pCR in the P arm (LR p=0.00029), and not the control arm (LR p=0.53), with a significant interaction with treatment (LR p=0.0064) that retains significance in a model adjusting for HR status. When dichotomized to optimize the biomarker x treatment interaction, the estimated pCR rate is 75% in P vs 18% in control, in the DDS+ subset.

Conclusion: In this small study, MP2 status and a DNA damage sensing pathway but not the PARPi7 or other repair pathways show promise as predictive biomarkers for immune checkpoint inhibition therapy in breast cancer.
2017 San Antonio Breast Cancer Symposium

Publication Number: PD7-01

Title: Impact of guideline concordant treatment on cost and health care utilization in early stage breast cancer patients

Courtney P Williams¹, Andres Azuero¹, Maria Pisu¹, Karina I Halilova¹, Stacey Adewakun¹, Supriya K Yagnik², Hans-Peter Goertz² and Gabrielle B Rocque¹. ¹University of Alabama at Birmingham, Birmingham, AL and ²Genentech, South San Francisco, CA.

Body: Introduction: National Comprehensive Cancer Network (NCCN) guideline-based treatment is increasingly recognized as a marker of high quality care. Payers are progressively limiting reimbursement for non-guideline based care as they move towards value-based cancer care. However, the impact of treatment regimen concordance with NCCN guidelines on cost and health care utilization in early stage breast cancer is unclear.

Methods: This was a secondary analysis of Medicare administrative claims data from 2012-2015 for all women aged ≥65 with stage I-III breast cancer who received care within the University of Alabama at Birmingham Cancer Community Network. Concordance to NCCN Clinical Practice guidelines was assessed for treatment regimens including hormonal medications, chemotherapy, and/or HER2-targeted therapy. Costs to Medicare (reimbursements to providers for all care received) were averaged monthly from the start of cancer treatment until death or available follow-up. Health care utilization (emergency department [ED] visits and hospitalizations) was identified from the start of cancer treatment until death or available follow-up. Cost and adjusted monthly utilization rates per thousand observations and their corresponding 95% confidence intervals (CI) were estimated using linear mixed effect models and generalized linear models, respectively, using the negative binomial distribution and log link function.

Results: Of 1042 patients on treatment for early stage breast cancer, 82% received a guideline concordant treatment, with 79% receiving a “preferred” treatment and 3% receiving an “other” but still on-guideline treatment. Those receiving guideline concordant treatment were more likely to be white, treated at large volume centers, have an earlier stage cancer, ER/PR positive, and HER2 negative (p<0.05). Among patients receiving chemotherapy (N=496), 63% of patients received guideline concordant treatment (55% “preferred”, 8% “other”). After adjusting for age, race, treatment center volume, and stage, average monthly costs after initiation of treatment for guideline concordant patients were $1464 lower compared to guideline discordant patients (95% CI $1135-$1793, p<.001). For guideline concordant patients, adjusted rates of ED visits per thousand observations were 41% lower at 51.3 per month (95% CI 44.0-59.8) compared to 77.9 per month (95% CI 62.6-96.9) for guideline discordant patients. Adjusted rates of hospitalizations per thousand observations were also 41% lower for guideline concordant patients at 28.2 per month (95% CI 23.5-33.8) compared to 42.7 per month (95% CI 32.9-55.5) for guideline discordant patients.

Conclusions: Despite the majority of early stage breast cancer patients receiving guideline concordant care, almost one in five did not, with an even higher proportion of guideline discordance in those receiving chemotherapy. Guideline concordant treatment was significantly associated with lower costs and lower rates of health care utilization after adjusting for patient and center characteristics. The appropriateness of guideline deviation should be examined from both the patient and payer perspectives.
**2017 San Antonio Breast Cancer Symposium**

**Publication Number:** PD7-02

**Title:** Value-based medicine: Are the 2013 guidelines for HER2 testing clinically significant and cost effective?

Anurag Sharma, Deepak Vadehra, Kaiser Talal, Poornima Hegde, Rong Wu and Susan Tannenbaum. 'University of Connecticut, Farmington, CT.

**Body:** Background: In the U.S., around 252,710 new breast cancer cases will be diagnosed in the year 2017. About 15-20% of these patients will be candidates for treatment with Her-2 directed therapy. The College of American Pathologists (CAP) published initial guidelines for immunohistochemistry (IHC) in 2003 with revisions in 2007 and 2013. 2013 guidelines define 3+(positive) as >10% intense complete membrane staining similar to 2003; >30% complete in 2007. Equivocal 2+ category in 2013 includes incomplete staining > 10% for the first time; this was negative in 2003 and 2007. Hypothesis: The 2013 guidelines do not result in more true positives but increased equivocal cases resulting in clinical uncertainty and increased cost. Methods: A retrospective analysis was performed of all IHC and FISH testing done at a single institution with a single pathology reader from 2003-2016. Criteria for IHC and FISH positivity were followed for each time period and compared to one another for positive and negative HER2 expression. The equivocal categories than compared for outcome by FISH. Ultimate numbers for percentage in each category compared for statistical significance. Results:

**IHC**

<table>
<thead>
<tr>
<th></th>
<th>Negative</th>
<th>Equivocal</th>
<th>Positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) 2003</td>
<td>279, 70.6%</td>
<td>71, 17.9%</td>
<td>45, 11.3%</td>
<td>395</td>
</tr>
<tr>
<td>(2) 2007</td>
<td>187, 71.6%</td>
<td>49, 18.7%</td>
<td>25, 9.5%</td>
<td>261</td>
</tr>
<tr>
<td>(3) 2013</td>
<td>181, 61.7%</td>
<td>79, 26.9%</td>
<td>33, 11.2%</td>
<td>293</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>647</td>
<td>199</td>
<td>103</td>
<td>949</td>
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<table>
<thead>
<tr>
<th>Chi-Square</th>
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<th>Value</th>
<th>Prob</th>
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<tr>
<td>Overall</td>
<td>4</td>
<td>10.4</td>
<td>0.03</td>
</tr>
<tr>
<td>Period 1 vs 2</td>
<td>2</td>
<td>0.5</td>
<td>0.75</td>
</tr>
<tr>
<td>Period 1 vs 3</td>
<td>2</td>
<td>8.2</td>
<td>0.01</td>
</tr>
<tr>
<td>Period 2 vs 3</td>
<td>2</td>
<td>6.4</td>
<td>0.04</td>
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</tbody>
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**IHC Equivocal Reflex to FISH**

<table>
<thead>
<tr>
<th></th>
<th>Negative</th>
<th>Equivocal</th>
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<td>(1) 2003</td>
<td>58, 82.8%</td>
<td>1, 1.4%</td>
<td>11, 15.7%</td>
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<tr>
<td>(2) 2007</td>
<td>34, 80.9%</td>
<td>0, 0.0%</td>
<td>8, 19.0%</td>
<td>42</td>
</tr>
<tr>
<td>(3) 2013</td>
<td>62, 82.6%</td>
<td>6, 8.0%</td>
<td>7, 9.3%</td>
<td>75</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>154</td>
<td>7</td>
<td>26</td>
<td>187</td>
</tr>
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</table>

<p>| | |</p>
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<tbody>
<tr>
<td><strong>DF</strong></td>
<td>4</td>
</tr>
<tr>
<td><strong>Chi-Square</strong></td>
<td>8.3</td>
</tr>
<tr>
<td>Asymptotic Pr &gt; ChiSq</td>
<td>0.08</td>
</tr>
<tr>
<td>Exact Pr ≥ ChiSq</td>
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</tbody>
</table>
When further analysis was carried out, period 1 and 2 were added together and compared to period 3. When all positives and negatives were compared, there was no statistical difference between the periods. However in the equivocal category, Period 1 and 2 were statistically different than period 3. In fact the only change in period 3 was the increase in the equivocal cases (same case-equivocal IHC and FISH).

**Conclusion:** In the 2013 CAP guidelines, 2+ IHC now includes incomplete staining in >10% of cells. This does not result in more positive cases as was the intention but an increase in the equivocal category by 8.19%. This adds to clinical uncertainty as to how to treat this group of patients. A send out for FISH is labor intensive, slow and costs on average of $650/case. With over 250,000 new cases expected this year in the U.S. this cost exceeds $13million. From all our data, the best parameters for IHC testing would be; positive-strong, complete staining in >10% cells and equivocal to eliminate the incomplete staining category. This would yield the highest number of true positives by FISH and almost eliminate ultimate equivocal cases.
Cost-effectiveness analysis of intraoperative radiotherapy for ductal carcinoma in situ

Maika Onishi1, Eileen P Connolly1, Jason D Wright1, Sowmya Vasan1, Tal Gross1, Wei-Yann Tsai1, Ling Chen1, Alfred I Neugut1, Melissa K Accordino1, Kevin Kalinsky1, Katherine D Crew1 and Dawn L Hershman1. 1Columbia University Medical Center, New York, NY.

Body: Background
Whole breast radiation therapy (WBRT) following lumpectomy for ductal carcinoma in situ (DCIS) is standard of care, however, the risk of local recurrence with and without radiation ranges as low as 0.9% vs. 6.7% over 7 years. Intraoperative radiotherapy (IORT) is a potential alternative with advantages of decreased toxicity to adjacent organs, convenience, and improved quality of life. While prospective trials of IORT for DCIS are ongoing, the objective of this study was to estimate the cost-effectiveness of IORT vs. WBRT vs. no radiation for DCIS.

Methods
We developed a Markov model using TreeAge Pro 2016 to evaluate the cost-effectiveness of WBRT, IORT, and no radiation in patients with DCIS following lumpectomy. Health states included disease free, local recurrence (ipsilateral DCIS or invasive cancer), distant recurrence or death due to breast cancer, and death due to non-breast cancer causes. A 10-year time horizon and societal perspective were used. Model input parameters were derived from the literature. Costs reflected 2016 Medicare rates. The primary endpoint was incremental cost-effectiveness ratio (ICER), defined as the difference in cost, divided by the difference in quality-adjusted life years (QALYs) of two interventions. We performed analyses of subgroups defined according to DCIS risk (histologic grade, Oncotype Dx® DCIS recurrence score, low risk per RTOG 9804 criteria) and endocrine therapy use (none, tamoxifen, aromatase inhibitor). Sensitivity analyses explored uncertainty in the model.

Results
IORT was the most cost-effective strategy, with an increase of 0.18 QALYs at an incremental cost of $4,728, corresponding to an ICER of $26,943/QALY when compared with no radiation therapy. WBRT resulted in an increase in 0.18 QALYs at an incremental cost of $6859, corresponding to an ICER of $39,085/QALY. For both strategies, the ICERs did not exceed the willingness to pay (WTP) threshold of $100,000.

IORT remained the most cost-effective strategy across DCIS risk groups, but was more cost-effective in higher risk patients, as demonstrated by lower ICERs. In low risk DCIS defined by RTOG 9804 criteria, no radiation was most cost-effective. The ICERs for IORT and WBRT, $152,753 and $208,204/QALY, respectively, exceeded the WTP threshold. IORT remained cost-effective in the setting of endocrine therapy use.

Incremental Cost-Effectiveness Ratios (ICER) for each radiation strategy for the base case and scenario analyses

<table>
<thead>
<tr>
<th>ICER ($/QALY)</th>
<th>No RT</th>
<th>IORT</th>
<th>WBRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base Case Analysis</td>
<td></td>
<td>26,943</td>
<td>39,085</td>
</tr>
<tr>
<td>Scenario Analysis by DCIS Risk Group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histologic Grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Low</td>
<td>36,811</td>
<td>52,219</td>
<td></td>
</tr>
<tr>
<td>- High</td>
<td>25,643</td>
<td>37,137</td>
<td></td>
</tr>
<tr>
<td>Oncotype Dx DCIS Score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Low</td>
<td>92,892</td>
<td>126,398</td>
<td></td>
</tr>
<tr>
<td>- High</td>
<td>32,003</td>
<td>45,690</td>
<td></td>
</tr>
<tr>
<td>Low Risk DCIS</td>
<td>152,753</td>
<td>208,204</td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>---------</td>
<td>---------</td>
<td></td>
</tr>
</tbody>
</table>

**Scenario Analysis by Endocrine Therapy**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cost 1</th>
<th>Cost 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Tamoxifen</td>
<td>23,387</td>
<td>34,373</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>47,811</td>
<td>66,616</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>31,961</td>
<td>46,272</td>
</tr>
<tr>
<td>Aromatase Inhibitor</td>
<td>41,316</td>
<td>58,674</td>
</tr>
</tbody>
</table>

**Conclusion**

IORT was the most cost-effective radiation strategy for DCIS compared to WBRT and no radiation. This applied to all subgroups with the exception of low-risk DCIS defined by RTOG 9804 criteria for whom no radiation was the most cost-effective strategy. These findings provide support for ongoing studies examining the role of IORT for DCIS with high-risk features, as well as alternative treatment strategies for low-risk DCIS.
Contribution of radiation therapy to the overall cost of cancer care for clinical stage IA breast cancer patients

Tomas Dvorak¹, Damien Coltey¹, John Waters¹, Patrick Kelly¹ and Daniel Buchholz¹. ¹Orlando Health, Orlando, FL.

**Body: Background:** Cost of cancer care continues to rise and there is an increasing interest in episode-of-care and bundled payments. Radiation therapy is a well-established component of clinical Stage IA breast cancer care. However, the contribution of radiation to the cost of breast cancer care is not well understood. We were interested in evaluating the contribution of radiation cost to our overall Cancer Center breast cancer care cost, by using revenue received as a proxy.

**Methods:** Patients with clinical Stage IA breast cancer managed completely at our Cancer Center between 1/1/2014 and 12/31/2014 were identified from cancer registry. An IRB-approved retrospective review of clinical charts and financial data of patients was performed. Individual chart review identified whether and which kind of surgery, chemotherapy, and radiation therapy was delivered. Financial review identified actual technical revenue received for 365 days after the date of first contact, and apportioned it accordingly to the various cost centers, including radiation oncology. All patients were included regardless whether they were Commercial, Medicare, Medicaid, self-pay, or free-care.

**Results:** There were 110 patients treated with clinical Stage IA breast cancer. All patients (100%) underwent surgery (lumpectomy 69%; mastectomy 5%; mastectomy with reconstruction 26%). Chemotherapy was delivered in 20% of patients (neoadjuvant 13%; adjuvant 7%). Seventy seven percent saw a radiation oncologist, and 57% received radiation therapy. Radiation technique was 3D in 92% and IMRT in 8%. Fractionation was conventional in 55%, accelerated in 43%, and APBI in 2%. Most common treatment pathways were lumpectomy with radiation (46%), mastectomy with reconstruction alone (18%), and lumpectomy alone (14%). Concordance with national guidelines was ≥90%. Overall cost of care for these patients was $6.3 million. Medicare accounted for 35% of patients. Radiation cost was $1.1 million (17%), compared with $2.1 million (33%) for surgery, $2.4 million (38%) for chemotherapy, and $0.7 million for other costs (imaging, lab, ED visits, etc).

**Conclusions:** Radiation therapy was used in ~60% of clinical Stage IA patients, but accounted for only ~20% of the overall cost to the health care system. Given the demonstrated survival benefit of radiation therapy in the care of breast cancer patients, and our high concordance with national guidelines, radiation therapy in our Cancer Center provides high value to our patients, as we move toward value-based episodes of care.
Title: Comparative costs of breast cancer screening with digital breast tomosynthesis versus digital mammography: A health system perspective

Geraldine J Liao¹, Henry A Glick², Marie B Synnestvedt², Mitchell D Schnall¹ and Emily F Conant¹. ¹Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA and ²Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA.

Body: Background
Digital breast tomosynthesis (DBT) is being rapidly implemented in breast cancer screening and demonstrates improved specificity and sensitivity compared to screening with digital mammography (DM) alone. Prior work based on payer perspectives has demonstrated that DBT can be cost-effective. However, DBT is costlier than DM, and there are little data from a health system perspective about the comparative test performance and costs of DBT versus DM.

Methods
We evaluated breast cancer screening episodes in a single health system between January 1, 2012 and December 31, 2013. A screening episode was defined as a single screening mammogram and all downstream breast diagnosis related costs for the following 1 year. Episodes were excluded if the patient had a prior diagnosis of breast cancer or reached 90 years of age before the end of the follow-up period. Test performance with respect to four outcomes – true positive (TP), true negative (TN), false positive (FP), and false negative (FN) rates – was determined by comparing the BI-RADS score assigned at screening with data about subsequent cancer diagnosis from institutional and state cancer registries. Cost data were developed using CPT codes collected from organizational billing systems and converted to the Medicare Physician Fee Payment Schedule for our region with an imputed additional charge of $60.16 for DBT. Based on this approach, a DM screening exam cost $155.66 and a DBT screening exam cost $215.82. We evaluated overall costs across a screening episode, as well as by four windows: screening, follow-up, diagnosis, and cancer treatment. Data were described using percentages, and Chi-squared and Fisher’s exact tests were used to evaluate differences in test performance outcomes and costs based on screening technology.

Results
There were a total of 46,483 cost episodes during the study period, of which 24,502 (52.7%) were screened by DM and 21,981 (47.3%) were screened by DBT. Overall, there were 224 TP (0.5%), 29 FN (0.1%), 4,530 FP (9.8%), and 41,700 TN (89.7%) episodes. Compared to DM episodes, DBT episodes had lower FP (8.6% vs. 10.8%, p<0.001) and higher TN (90.9% vs. 88.7%, p<0.001) rates. There were no statistically significant differences between DBT and DM episodes with respect to TP and FN rates.

Overall, average episode costs were higher for DBT compared to DM ($378.02 vs. $286.62, p<0.001). This $91.40 difference was driven by higher average screening costs ($215.94 vs. $155.76, p<0.001), which approximated the additional charge for DBT, as well as follow-up costs ($23.67 vs. $12.11, p<0.001). There was no significant difference in costs between DBT and DM episodes with respect to TP and FN rates.

Compared to DM episodes, DBT episodes had equivalent average episode costs per woman screened for FP ($67.75 vs. $65.71, p=0.49), FN ($4.63 vs. $5.60, p=0.69) and TP ($85.80 vs. $65.15, p=0.07) outcomes, but higher costs for TN ($219.84 vs. $150.16, p<0.001) outcomes.

Conclusion
At a single health system, screening with DBT decreased FP rates and increased TN rates compared to screening with DM. DBT costs more overall, but not on a per-woman-screened basis for FP, FN, and TP outcomes.
Title: MAAT: Menses after adjuvant treatment. Prediction of menses recovery after chemotherapy for early breast cancer (BC) by using a nomogram model in UNICANCER PACS04 and PACS05 trials

Barbara Pistilli¹, Chafika Mazouni², Anna Zingarello¹,⁵, Matthieu Faron²,³, Mahasti Saghatchian¹, Michael Grynberg⁴, Marc Spielmann¹, Pierre Kerbrat⁶, Henri Roché⁷, Veronique Lorgis⁸, Thomas Bachelot⁹, Mario Campone¹⁰, Christelle Levy¹¹, Anthony Goncalves¹², Anne Lesur¹³, Corinne Veyrat¹⁴, Laurence Vanlemmens¹¹, Jerome Lemonnier¹⁶ and Suzette Delaloge¹. ¹Institut Gustave Roussy, Villejuif, France; ²Institut Gustave Roussy, Villejuif, France; ³Institut Gustave Roussy, Villejuif, France; ⁴Institut Gustave Roussy, Villejuif, France; ⁵IRCCS AOI San Martino - IST, Genova, Italy; ⁶Centre Eugene Marquis, Rennes, France; ⁷Institut Claudius Regaud, IUCT-OnCopole, Toulouse, France; ⁸Centre Georges François Leclerc, Dijon, France; ⁹Centre Léon Bérard, Lyon, France; ¹⁰Centre, Institut de Cancérologie de l’Ouest, Angers, France; ¹¹Centre F Baclesse, Caen, France; ¹²Institut Paoli-Calmettes, Marseille, France; ¹³Institut de Cancérologie de Lorraine, Nancy, France; ¹⁴Centre Henri Becquerel, Rouen, France; ¹⁵Centre O Lambret, Lille, France and ¹⁶UNICANCER, Paris, France.

Body: Purpose: The likelihood of menses recovery (MR) is largely variable in premenopausal patients (pts) receiving adjuvant chemotherapy for BC. Quantifying this probability for each single patient could impact discussion of chemotherapy side effects and better individualize fertility counseling. We performed a pooled analysis from PACS04 and PACS05 randomized trials aiming to develop a nomogram to estimate the probability of menses recovery at 6 and 18 months (mos) after the end of adjuvant chemotherapy (CT) for premenopausal pts with early BC.

Patients and Methods: The analyzed population consisted of 1683 pts who were premenopausal and ≤ 50 (out of 4524 enrolled in both trials). In PACS05 node-negative BC pts were randomized to 4 or 6 cycles of FE_{100}C (standard arm); in PACS04 node-positive pts were randomized to 6 cycles of FE_{100}C or 6 cycles of Epirubicin 75mg/m² and Docetaxel 75 mg/m² (ED75). Endocrine therapy (ET) (Tamoxifen) x 5 years was mandatory for ER+ BC. Variables significantly associated with MR in the univariate analysis (P<0.20) were included in the multivariate analysis. Using this data set, a logistic regression-based nomogram was developed to predict MR at 6 and 18 mos.

Results: Pts’ characteristics were: median age 43 (22-50), median body mass index (BMI) at baseline 22.6 (15.6-54.7), at the end of chemotherapy 22.8 (15.8-58.6). ED75 was administrated to 517 (30.7%), while 802 (47.7%) received 6FE_{100}C, 364 (21.6) 4FE_{100}C. Trastuzumab was given to 122 (7.2%), ET to 1229 (73%) pts. CT-induced amenorrhea was observed in 1407 (83.6%) pts. Factors associated to MR were assessed on 1210 pts (excluding pts who recovered menses during CT or of whom date of recovery was not specified). At a median follow-up of 90 mos, 28.2% (342/1210) of pts had recovered menstrual cycles: 11% (133/1210) at 6 mos and 24.3% (294/1210) at 18 mos. Multivariate analysis showed that younger age, higher BMI at the end of CT, non-alkylating agents and absence of ET were independently associated to MR.

Table1 Multivariate Cox regression analysis of menses recovery

<table>
<thead>
<tr>
<th>Variables</th>
<th>HR (95%CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.49 (1.16-1.93)</td>
<td>&lt; 0.002</td>
</tr>
<tr>
<td>Age²*</td>
<td>0.99 [0.98-0.99]</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI after CT</td>
<td>1.02 (0.99-1.04)</td>
<td>0.07</td>
</tr>
<tr>
<td>Alkylating agents</td>
<td>0.72 (0.57-0.90)</td>
<td>0.004</td>
</tr>
<tr>
<td>Endocrine Therapy</td>
<td>0.50 (0.40-0.62)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* The quadratic term in the age variable accounts for the non-linearity of the relation between the age and the probability of recovering menses. Overall this probability tend to decrease when age increase with a greater decrease for the older patients.

Nomogram concordance-index was 0.749 and 0.750 for predicting MR at 6 and 18 mos respectively. A better calibration was observed at 18 mos, comparing nomogram predictions with the actual probability of MR in the 1210 women.

Conclusion: Our analysis confirmed the possibility of developing a user-friendly nomogram for predicting menses recovery after adjuvant chemotherapy. As next step, we will externally validate our nomogram on CANTO premenopausal population, one of the
biggest national cohorts aiming to assess the long-term impact of cancer treatments toxicities (*UNICANCER* NCT01993498 - http://etudecanto.org/).
Title: Failure of goserelin to prevent chemotherapy-induced damage to ovarian reserve

Kutluk Oktay1,2, Enes Taylan1,2, Yodo Sugishita3, Tai Kawahara1,3 and Nao Suzuki3. 1Yale University School of Medicine, New Haven, CT; 2Innovation Institute for Fertility Preservation and IVF, New York, NY and 3St. Marianna University School of Medicine, Kawasaki, Japan.

Body: Objective
The effectiveness of GnRH analogs (GnRHa) in preserving ovarian reserve and fertility against chemotherapy-induced damage is still being debated. Prior studies generally used menstruation as an outcome measure, which is an unreliable surrogate for fertility. Cyclophosphamide is one of the key components of most commonly utilized adjuvant and neo-adjuvant chemotherapy protocols in breast cancer. Ovarian reserve is made up of quiescent primordial follicles, which are irreversibly damaged by gonadotoxic chemotherapy agents such as cyclophosphamide (Cy) via the induction of double strand DNA breaks (DSBs) that trigger apoptotic oocyte death (Soleimani et al, Aging 2011). There is currently no data on whether GnRHa co-treatment would block chemo-induced primordial follicle DNA damage. We conducted this animal study to determine whether co-administration of a GnRHa would protect cyclophosphamide-induced primordial follicle death and DNA damage. If ovarian suppression by GnRHa does not protect chemotherapy-induced damage to ovarian reserve, then there would be no biological basis for utilizing this strategy for fertility preservation.

Design
Experimental animal study.

Materials and Methods
After unilateral oophorectomy, 4-week-old FVB mice received daily 200 mg/kg Cy injections either alone for 3 days (n=4) or with 250 µg/kg Goserelin co-treatment (n=4) starting 3 days before and continuing the duration of chemotherapy. The remaining ovary was recovered at the end of chemotherapy for analysis. Ovaries were fixed and serially sectioned at 5 µm, and every 10th section was analyzed. Primordial (pdf) density, oocyte DSBs (by γH2AX) and apoptotic cell death pathway activation (AC3) were determined by immunostaining and compared between the pre- and post-chemo ovaries of each mice in a paired analysis, as well as between the 2 groups post-chemo.

Results
Results are summarized in table-1. In both the Cy-only and Cy+GnRHa groups, pdf densities declined significantly compared to pre-chemo (p= 0.003). However, there was no difference between the post-chemo pdf densities of the two groups. Likewise, both Cy and Cy+GnRHa resulted in significantly increased oocyte DNA damage and apoptotic death (p<0.01) compared to baseline readings. However, post-chemo incidence of oocyte DNA damage and apoptotic death were similar between the Cy and Cy+GnRHa groups (p= 0.69 and 0.78, respectively).

Impact of GnRHa on Chemo-Induced Ovarian Damage

<table>
<thead>
<tr>
<th></th>
<th>Pre-chemo</th>
<th>Cy</th>
<th>Cy+GnRHa</th>
<th>Cy vs. Pre-chemo</th>
<th>Cy+GnRHa vs. Pre-chemo</th>
<th>Cy vs. Cy+GnRHa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pdf Density (/mm3)</td>
<td>1,300±25.8</td>
<td>685±128.4</td>
<td>640±121.3</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.8</td>
</tr>
<tr>
<td>γ-H2AX+ Pdf (%)</td>
<td>12.3±0.08</td>
<td>71.2±6.9</td>
<td>75±5.8</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.69</td>
</tr>
<tr>
<td>AC3+ Pdf (%)</td>
<td>0</td>
<td>53.6±0.6</td>
<td>38.1±0.06</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.78</td>
</tr>
</tbody>
</table>

Pdf: primordial follicle. Data are presented as mean ±SE. P < 0.05 considered as statistically significant.

Conclusions
This animal study provides the first direct histologic and molecular evidence that GnRHa co-treatment does not prevent chemotherapy-induced damage to ovarian reserve including potentially mutagenic DNA DSBs in oocytes. Taken together with the recent clinical data utilizing more reliable ovarian reserve markers such as the anti-mullerian hormone (Demeestere et al, J Clin Oncol, 2016), ovarian suppression via GnRHa should not be recommended for the protection of ovarian reserve. (Supported by
NIH RO1 HD053112).
Title: Economic and time-related burden in young breast cancer patients wishing to preserve their fertility before undergoing cancer treatment

Atsusko Kitano\textsuperscript{1,2}, Mie Mifune\textsuperscript{1}, Aoi Suzuki\textsuperscript{1}, Aya Hashizume\textsuperscript{1}, Chie Nakayama\textsuperscript{1}, Yasuyo Motofuji\textsuperscript{1}, Hitomi Suzuki\textsuperscript{1,3} and Emika Ichioka\textsuperscript{1}. \textsuperscript{1}Pink Ring, Tokyo, Japan; \textsuperscript{2}School of Public Health, St Luke International University, Tokyo, Japan and \textsuperscript{3}Ichinomiyanishi Hospital, Ichinomiya, Japan.

Body: Background: Treatment-related infertility is one of many issues facing young breast cancer patients. Fertility preservation, using artificial reproductive therapy (ART), is a recommended method to maintain future reproductive function among cancer patients. However, medical costs for fertility preservation are often too expensive for young cancer patients who can be less economically secure, in addition to the costs of cancer treatment. Moreover, the ART process requires several weeks or more, and time spent in fertility preservation may potentially delay treatment. Pink Ring is a patient advocacy group for young breast cancer patients, established in Japan in 2012, which has been working on the issue of onco-fertility. We conducted a cross sectional web-based survey exploring the economic and time-related burden of young cancer patients in the reproductive age group. This study was supported by a research grant from the Foundation to Promote Cancer Research.

Method: Pink Ring conducted a web-based survey, and 343 young breast cancer patients who responded were enrolled. We performed descriptive analysis of the data.

Result: Among 343 young breast cancer patients, the mean age at the time of the survey was 38 years (20-49) and the mean age at breast cancer diagnosis was 34 years (20-49). At diagnosis, 167 (49\%) of the women were married and 171 (50\%) were single. At diagnosis, 99 (29\%) women had had a child or children and 236 (63\%) were childless. Regarding treatment-related infertility, 193 patients (56\%) had had discussions with a medical health provider. However, 58 (17\%) patients underwent a fertility preservation procedure. Among patients who underwent fertility preservation, 35 patients (60\%) underwent embryo preservation, 23 (40\%) underwent oocyte cryopreservation, and 4 (7\%) underwent ovarian tissue cryopreservation. According to medical payment for a fertility preservation procedure, 26 patients (45\%) paid less than $5000, 21 patients (36\%) paid between $5000 and $10000 and 10 patients (17\%) paid over $10000. Regarding cost-effectiveness, 51 patients (88\%) considered that the medical payment was expensive. According to the duration of the fertility preservation procedure, 7 patients (12\%) were treated up to 2 weeks, 8 patients (31\%) were treated between 2 and 4 weeks, 21 patients (36\%) were treated between 1 and 2 months, and 7 patients (12\%) were treated longer than 2 months. Twenty-eight patients (48\%) answered that planned cancer treatment was delayed because of a fertility preservation procedure. According to cancer treatment delay, 4 patients (14\%) were delayed up to 4 weeks, 14 patients (50\%) were delayed between 1 and 2 months, and 10 patients (36\%) were delayed longer than 2 months. Among 285 patients who did not proceed with fertility preservation, the major reasons given for their decision were as follows: lack of awareness regarding treatment-related infertility and fertility preservation options (29\%), economic-related burden (25\%), and time-related burden (25\%).

Discussion: This survey revealed that fertility preservation was a burden not only for economic reasons but also in terms of time required for treatment for young breast cancer patients of reproductive age.
Body: Background:

We previously demonstrated that the majority of women ≤45 years experienced chemotherapy-induced ovarian failure (CIOF) after CT for EBC. Age, CT regimen, duration and dose-density influenced the rate of CIOF. The regain of premenopausal Follicle-Stimulating Hormone (FSH) and Estradiol (E2) levels after chemotherapy is not equivalent to fertility restoration. The Anti-Muellerian Hormone (AMH) assessment seems to be more accurate than other hormones in predicting the ovarian reserve. FSH, E2 and AMH have been prospectively assessed in young patients receiving (neo)adjuvant CT.

Methods:

740 patients (pts) aged ≤45yrs treated with anthracycline or taxane-based CT for EBC from 4 German neoadjuvant/adjuvant trials were included. Blood samples were collected at baseline before CT (N=740), end of treatment (EOT n=740), 6 (n=177), 12 (n=113), 18 (n=69), 24 (n=47) months (m) after EOT. Only the full set of samples of a given patient was included. FSH, E2 and AMH were centrally assessed. Postmenopausal hormone levels of FSH and E2 according to the central laboratory were defined as FSH>12.4IU/l and E2<52.2ng/l; fertile level of AMH as ≥0.22ng/ml. Regain of premenopausal hormone levels was defined as the time point from EOT to premenopausal FSH and E2 level regain and was assessed only for those pts with postmenopausal FSH and E2 levels at EOT. Pts with no regain have been censored at the date of the last hormone assessment.

Results:

Median age was 40yrs (range 21-45); 57.2% had BMI 18.5-<25, 41.1% ≥25; 32% had luminal-like, 35.9% HER2+, 32.0% triple-negative BC. Median hormone levels at different time points are presented in Table 1. Before chemotherapy 14.2% of pts had non-fertile hormone levels of AMH despite premenopausal levels of FSH and E2 compared to 77.3% of pts with postmenopausal hormone levels of AMH as ≥0.22ng/ml. Regain of premenopausal hormone levels was defined as the point from EOT to premenopausal FSH and E2 level regain and was assessed only for those pts with postmenopausal FSH and E2 levels at EOT. Pts with no regain have been censored at the date of the last hormone assessment.

Conclusion:

Nearly 70% of women regain premenopausal hormone levels of FSH and E2 within 2 years after end of CT. Despite that, only less than one third maintain their fertility potential as predicted by AMH. AMH is a very sensitive marker for the prediction of fertility function after CT for EBC.
<table>
<thead>
<tr>
<th>Age (m)</th>
<th>Value 1 [Range]</th>
<th>Value 2 [Range]</th>
<th>Value 3 [Range]</th>
<th>Value 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>28.7 [1.1-146.0]</td>
<td>11.0 [dt-947.0]</td>
<td>dt [dt-2.81]</td>
<td>29.2</td>
</tr>
<tr>
<td>18</td>
<td>20.6 [0.8-172.3]</td>
<td>19.0 [dt-624.0]</td>
<td>dt [dt-1.89]</td>
<td>34.8</td>
</tr>
<tr>
<td>24</td>
<td>16.30 [dt-93.9]</td>
<td>44.0 [dt-11795.0]</td>
<td>dt [dt-1.75]</td>
<td>38.3</td>
</tr>
</tbody>
</table>

Abbreviations: dt, detectable threshold; EOT, end of treatment; m, month; pts, patients. Detectable threshold: FSH<0.1IU/l, E2<5ng/l, AMH<0.03ng/ml
**Title:** Male-GBG54: A prospective, randomised multi-centre phase II study evaluating endocrine treatment with either tamoxifen +/- gonadotropin releasing hormone analogue (GnRHa) or an aromatase inhibitor + GnRHa in male breast cancer patients

M Reinisch¹, S Seiler², T Hauzenberger⁴, S Schmatloch⁵, HJ Strittmatter⁶, DM Zahm⁷, C Thode⁸, C Jackisch², J Furlanetto⁹, D Strik¹⁰, E Stiekeler¹⁴, F Marmé¹⁵, W Janni¹⁶, M Schmidt¹⁷, A Kamischke¹⁸, C Rudlowski¹⁹, V Nekljudova¹, G von Minckwitz³ and S Loibl³.

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**Body: Background**
Over 90% of male breast cancer (BC) patients (pts) have hormone receptor (HR) positive disease. Until today tamoxifen is considered to be the standard of care in male BC pts. Due to the low incidence of male BC, there is a lack of data regarding efficacy and safety. Therapeutic strategies are extrapolated from the standard of care of female BC. However, no prospectively randomized study in male BC pts has been conducted so far. The Male-GBG54 study is the first prospective, randomized, multicenter trial evaluating the efficacy and safety of different endocrine treatment options in male BC patients.

**Patients and Methods**
In the phase II Male-GBG54 trial (NCT01638247), pts were randomized to receive either tamoxifen 20 mg/day per os (p.o.) or tamoxifen 20mg/day p.o. + GnRHa subcutaneous (s.c.) q3m or exemestane 25 mg/day p.o. + GnRHa s.c. for 6 months as (neo)adjuvant or metastatic therapy. Further treatment was conducted as per local guidelines. Primary objective was the suppression of oestradiol in the three treatment arms after 3 months of therapy. Secondary objectives comprised oestradiol suppression after six months and safety of the three therapies, the level of different steroidal hormones (testosterone, dihydrotestosterone, SHBG, FSH, LH) and osteocalcin in the three arms. Quality of Life was assessed using the Aging Male Symptom Score, International Index of Erectile function and International Prostate Symptom Score. Tissue and blood was collected for translational research. Male BC pts with a Karnofsky Index ≥60%, normal blood lipids, and no history or evidence of prostate cancer were eligible. 14 pts per group were needed for the F-test to have 80% power to detect a difference in mean oestradiol decrease between the three therapeutic groups at the 5% significance level. The final sample size was calculated as 48 patients, as a non-parametric test (Kruskal-Wallis) was chosen in case the oestradiol levels are not normally distributed within the study population.

**Results**
Between October 2012 and May 2017, 55 pts were randomized within 24 centers in Germany. 52 pts were included in the adjuvant and 2 pts in the metastatic setting of whom 48 pts were fully evaluable and comprised the analysis set. The median age was 62 years (range 37-83 years). The baseline characteristics were well balanced between the 3 treatment groups. Karnofsky Index ranged from 60%-100%, 36.4% had received prior chemotherapy and 12.7% had HER2-positive BC. The majority had a pT2 (52%), pN0 (58.0%) BC. 3 serious adverse events were reported, none was related to the study treatment.

**Conclusion**
This is the first prospective randomized trial worldwide evaluating the impact of three different endocrine treatments in male breast cancer. The therapy was well tolerated without safety signals. At the meeting, final results of the primary and secondary endpoints will be present.
**2017 San Antonio Breast Cancer Symposium**

**Publication Number:** PD7-11

**Title:** The role of multi-gene hereditary cancer panels in male patients with breast cancer

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**Body:**

**Background/Statement of Purpose:** The role of cancer susceptibility genes in the male breast cancer population beyond BRCA1 and BRCA2 (BRCA) is not well defined. While breast cancer has been documented in men with pathogenic variants in a number of other breast cancer susceptibility genes (e.g. CHEK2, PALB2, PTEN), the yield of testing is not well documented nor are predictive clinical features of those likely to harbor causative variants. This study assesses the yield of pathogenic/likely pathogenic variants (collectively, PV) in male breast cancer patients who underwent multi-gene hereditary cancer panel testing. In addition, we aim to examine predictors of identifying a PV in this population.

**Methods:** Clinical histories and test results were reviewed for men with a diagnosis of breast cancer who underwent panel testing that included a minimum of eight well-described breast cancer susceptibility genes (ATM, BRCA1, BRCA2, CDH1, CHEK2, PALB2, PTEN, TP53) and up to 24 additional genes. Using t-test and two-tailed Fisher's exact test, we assessed whether age at diagnosis, family history of breast cancer, or the presence of selected second primary cancers (second breast, prostate, pancreatic, colon, or melanoma cancers) were associated with a greater likelihood of identifying a PV.

**Results:** The clinical histories and test results of 381 men with breast cancer were reviewed, of whom 12.1% had at least one PV (46/381). When we limited our assessment to men who had not had prior negative BRCA testing, 13.3% had at least one PV (42/315). Variants were most commonly detected in BRCA2 (21) and CHEK2 (17), followed by PALB2 (4), BRCA1 (4), and ATM (2). A two sample t-test showed no significant difference (p=0.39) in the average age of diagnosis for those with a PV (62.5y, n=47) compared to those without a PV (60.9y, n=334). Two-tailed Fisher's exact test showed no association between having a PV and a history of a selected second primary (SP) cancer [10.8% (7/65) w/SP vs 12.3% (39/316) w/out SP; p=0.84]. Lastly, two-tailed Fisher's exact test showed those with a family history of breast cancer (fhx br) were more likely to have a PV [15.1% (32/212) fhx br vs. 8.3% (14/169) no fhx br], although this did not reach statistical significance (p=0.06).

**Conclusions:** While BRCA2 remains the most common gene in which PVs are identified in men with breast cancer, a significant proportion of patients will have a PV in another well-described breast cancer susceptibility gene, particularly CHEK2, PALB2, and ATM. Therefore, it is reasonable to utilize a panel that is inclusive of these genes when testing male breast cancer patients. As the likelihood to harbor a PV was not significantly associated with age of onset, family history of breast cancer, or presence of a second primary, all men with breast cancer could consider genetic testing. Further study is warranted as the current sample size may limit the power to detect associations.
**Title:** Molecular subtyping of male breast cancer by the International male breast cancer program (IMBC): EORTC 10085/TBCRC 0-29/BIG 2-07/NABCG/BOOG 2009-04


**Body:** Introduction. Male breast cancer (male BC) is a rare disease for which disease management is extrapolated from females. IMBC, an international consortium, which previously reported on clinico-pathological aspects, now reports on molecular subgroups revealed by RNA sequencing and their relation to patient outcome.

**Methods.** Tumor samples from the retrospective MALE BC registry diagnosed between 1990-2010 and with pathology and outcome data (relapse-free- (RFS) and overall survival (OS)) were included (n=699). To allow the discovery of prognostic features, we selected, stratified for known risk factors (TN stage, grade, age at diagnose and adjuvant endocrine treatment), from the cohort 152 cases with poor (RFS <= 4 yrs) and good outcome (RFS > 7yrs) evenly distributed. Here, we report RNA sequencing results of the first 73 cases, 38 with poor and 35 with good outcome. RNA sequencing reads were used to generate gene expression values and to report transcripts carrying driver mutations. Unsupervised clustering identified subgroups and their relation to patient outcome.

**Results.** Unsupervised clustering revealed 2 main subgroups of which group 1 was enriched for expression of ER target genes, WNT3 and genes from amplicons known for female BC, e.g. 19p13 (CCNE1), 8q24 (MYC), and 11q13 (CCND1). The biology of the smaller group 2 was less defined but TGFβ2 expression was high as were various kallikreins (KLK) and WNT3 and genes from amplicons known for female BC, e.g. 19p13 (CCNE1), 8q24 (MYC), and 11q13 (CCND1). The biology of the smaller group 2 was less defined but TGFβ2 expression was high as were various kallikreins (KLK) and WNT3 and genes from amplicons known for female BC, e.g. 19p13 (CCNE1), 8q24 (MYC), and 11q13 (CCND1).

**Conclusions.** The previously reported M2 subgroup, which largely segregated with subclusters 1a and 1b, was associated with a better RFS than the M1 subgroup (OR=2.9; 95%CI 1.1-7.5;
p-value=0.03).

**Conclusions.** 1) Intrinsic subtypes of male BC were revealed and their subgrouping is defined by ER associated subsets of genes. 2) The association of the reported M2 subgroup of male BC with longer RFS was validated; 3) Currently identified biological characteristics of male BC may improve future treatments. The full report on 152 cases including a comparison to female BC will be presented at the conference.

This research was funded by Breast Cancer Research Foundation
Title: Molecular characterization and mortality from breast cancer in men

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Body: Background: Limited data exist on the molecular biology, treatment, and outcomes of breast cancer in men and much of our understanding in this area remains largely an extrapolation from data in women with breast cancer.

Methods: We studied men and women with hormone receptor (HR)-positive breast cancer and 21-gene test (RS) results. Patients with negative nodes, micrometastasis, and 1-3 positive nodes were included. Differences in clinical characteristics and gene expression were determined and distribution of RS results was analyzed and correlated with 5-year breast cancer specific survival (BCSS) and overall survival (OS).

Results: There were 3806 men and 571115 women. Men were older than women (mean age 64.2 vs. 59.1 years, p<0.001). RS <18 predominated in both genders, but RS ≥31 was more frequent in men (12.4% vs. 7.4%, p<0.001) as were very low scores (RS <11) (33.8% vs. 22.1%, p<0.001). Mean gene expression was higher in men for the ER, proliferation, and invasion groups. ER was lowest and PR highest in women <50 years, with a progressive increase in ER with age. Men <50 years had slightly lower ER and PR compared to older men. Survival data was available from SEER for 322 men and 55842 women. 5-year estimates for BCSS differed significantly between RS groups for both men (p=0.003) and women (p<0.001). For men, the 5-year BCSS was 99.0% and 95.9% with RS <18 and RS 18-30, respectively, and for women it was 99.5% and 98.6%, regardless of nodal status. RS ≥31 was associated with a 5-year BCSS of 81.0% in men and 94.9% in women. The prognostic utility of RS was evident in both men and women, despite the progressive increase in adjuvant chemotherapy use with higher RS results. 5-year BCSS and OS were overall lower in men than in women.

Conclusion: This large genomic study reveals some distinctive biologic features of breast cancer in men and an important prognostic role for 21-gene testing in both men and women, regardless of nodal status. Future adjuvant trials in ER-positive breast cancer should focus on targeting endocrine-resistance in those patients with RS ≥31, and need to consider the weight of competing causes of mortality when investigating the value of any additional treatment beyond endocrine therapy.
Body: Background: Typically defined by negative IHC staining for E-cadherin, classic (CILC) and pleomorphic (PILC) are often combined as a single breast cancer subtype. We queried whether patients with relapsed metastatic disease, mCILC and mPLIC, would harbor contrasting genomic alterations (GA) and that molecular information could further differentiate the 2 tumor types and thereby influence therapy selection.

Methods: DNA was extracted from 40 µm of FFPE sections of 10,784 invasive breast carcinomas. 454 (4%) CDH1 mutated mILC were selected including 428 classic mCILC (94%) and 26 mPLIC (6%) subtypes. Comprehensive genomic profiling (CGP) was performed on hybridization-captured, adaptor ligation-based libraries to a mean coverage depth >600X for up to 315 cancer-related genes. Tumor mutational burden (TMB) was determined on 1.1 Mbp of sequenced DNA.

Results: mCILC and mPLIC patients featured a median age of 63 years (Table). Slide based ER+ status and HER2+ status was significantly different in both groups (P<0.0001). The frequency of base substitutions in ESR1 was significantly higher in mCILC, and this difference was also significantly higher in mCILC metastasis biopsies exposed to hormonal therapy than in pre-treatment primary tumors (P<0.0001). ERBB2 (HER2) GA (amp + non-amp) detected by CGP were higher in mPLIC than mCILC in both pre-and post-treatment samples (P<0.0001 for both). The ERBB2 GA frequency was nearly twice as high after hormonal therapy in both mCILC and mPLIC. ESR1 and ERBB2 GA were mutually exclusive overall and especially in the mCILC group. PIK3CA GA were the most frequent GA in both mCILC and mPLIC. TP53 GA were significantly more frequent in mPLIC than mCILC. At 19%, the frequency of TMB > 15 mutations/Mb in mPLIC was more than twice as frequent than in mCILC (P=0.046). All (100%) of both the CILC and PILC groups were negative for mis-match repair deficiency or MSI high status. mCILC and mPLIC patients with post primary therapy associated ESR1 and ERBB2 GA responding to targeted and immunotherapies will be presented.

Contrasting Clinical and Genomic Features of CILC and PILC

<table>
<thead>
<tr>
<th></th>
<th>Classic CILC (428 cases)</th>
<th>Pleomorphic PILC (26 cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median Age</td>
<td>63</td>
<td>63</td>
</tr>
<tr>
<td>*ER+</td>
<td>98%</td>
<td>74%</td>
</tr>
<tr>
<td>*HER2 IHC/FISH+</td>
<td>12 (3%)</td>
<td>6 (22%)</td>
</tr>
<tr>
<td>ESR1 GA Primary Pre-Rx</td>
<td>6%</td>
<td>0%</td>
</tr>
<tr>
<td>ESR1 GA Metastatic Post-Rx</td>
<td>17%</td>
<td>0%</td>
</tr>
<tr>
<td>ERBB2 GA Primary Pre-Rx</td>
<td>7%</td>
<td>18%</td>
</tr>
<tr>
<td>ERBB2 GA Metastatic Post-Rx</td>
<td>12%</td>
<td>34%</td>
</tr>
<tr>
<td>Other Significant GA</td>
<td>PIK3CA (55%), CCND1 (21%), TP53 (17%), ARID1A, AKT3, MDM4, PTEN (all 11%)</td>
<td>PIK3CA (58%), TP53 (30%), AKT1 22%, FGFR4, CCND1, PTEN (all 17%)</td>
</tr>
<tr>
<td>TMB median (mut/Mb)</td>
<td>2.7</td>
<td>3.6</td>
</tr>
<tr>
<td>TMB &gt; 15%</td>
<td>8%</td>
<td>19%</td>
</tr>
</tbody>
</table>
Conclusions: CGP of mCILC and mPILC reveals significant differences in the panorama of GA both in pre-treatment primary and metastatic disease lesions especially in therapy-impacting GA in *ESR1* and *ERBB2*. mCILC is more often driven by *ESR1* GA and mPILC by *ERBB2* GA. Although both mCILC and mPILC feature subsets of tumors with high TMB, this is more frequent for mPILC likely indicating different potentials for immunotherapies to benefit these patients.
Title: Mechanisms of recurrence: Paired analysis of primary and metastatic triple negative breast cancer

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Body: Background: Triple negative breast cancer (TNBC) is the most aggressive subtype of invasive breast cancer that lacks ER, PR, and HER2 expression. It is a heterogeneous disease with several molecular subtypes: basal-like1 (BL-1), basal-like 2 (BL-2), mesenchymal (M), and luminal androgen receptor (LAR). Treatment for TNBC is normally limited to chemotherapy, and relapse is common. Here we report molecular alterations associated with TNBC metastasis by analyzing the genomic profiles of paired primary and metastatic TNBCs.

Methods: 50 paired TNBCs were identified through an IRB-approved protocol via the City of Hope (COH) Biospecimen Repository. DNA mutation and mRNA expression profiles of 10 paired primary and metastatic TNBCs were analyzed. DNA mutations were identified using exome sequencing by FoundationOne®. Affymetrix Human Genechip 2.0 was used for mRNA expression profiling. Raw data were normalized and processed using Expression Console, and linear regression was performed using Limma to identify the differentially expressed genes between primary and metastatic TNBCs.

Results: DNA mutation profiling showed that multiple mutations occurred within genes covering pathways of PI3K/AKT/mTOR, DNA repair, RAS/MAPK, cell cycle, and growth factor receptor signaling, reconfirming genomic heterogeneity of TNBCs. Gene expression profiles were analyzed for Lehmann's TNBC molecular subtypes (BL-1, BL-2, M, and LAR). Six of ten TNBCs showed phenotype shift between the primary and metastatic TNBCs. Several unique gene expression patterns were identified by comparing the paired TNBCs. CCNE1 and TPX2 were co-overexpressed in metastatic TNBCs compared to paired primaries. This mirrored prior studies in ovarian cancer, where co-overexpression of CCNE1 and TPX2 were found related to clonal resistance against chemotherapy. Splicing factors TRA2B and SRSF7 were also over-expressed in metastatic TNBCs compared with primaries. The analysis studying the association of CCNE1 and TPX2 with overall survival is ongoing using TCGA.

Conclusion: Overall, these results show the comparative changes between primary and relapsed TNBCs and indicate the heterogeneity of molecular mechanisms of recurrence. CNNE1 and TPX2 may represent important genes involved in TNBC metastasis. Further analyses including a total of 50 paired TNBCs are currently underway.

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ESR1 gene fusions drive endocrine therapy resistance and metastasis in breast cancer

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**Body: Background.** Dysregulation of the estrogen receptor gene (ESR1) is an established mechanism of inducing endocrine therapy resistance. We previously discovered a chromosomal translocation event generating an estrogen receptor gene fused in-frame to C-terminal sequences of YAP1 (ESR1-YAP1) that contributed to endocrine therapy resistance in estrogen receptor positive (ER+) breast cancer models. This study compares functional, transcriptional, and pharmacological properties of additional ESR1 gene fusion events of both early stage (ESR1-NOP2) late stage (ESR1-YAP1 and ESR1-PCDH11x) breast cancers to gain a better understanding of therapeutic resistance and metastasis. Understanding the role of ESR1 fusions in inducing metastasis is critical, since the primary cause of death in breast cancer patients is through metastasis to distant sites.

**Methods.** RNA-seq screens identified ESR1 fusions from early and late stage, endocrine therapy resistant breast tumor samples. Functional experiments were conducted using ER+ breast cancer cell lines, xenograft, and PDX models to test the ability of ESR1 fusions to induce therapeutic resistance and metastasis. ChIP-seq and RNA-seq were performed to examine transcriptional properties and differential gene expression induced by the fusions which directed subsequent pharmacological experiments with a CDK4/6 inhibitor.

**Results.** ESR1-YAP1 and ESR1-PCDH11x promoted estrogen-independent and fulvestrant-resistant growth in vitro and induced greater tumor growth and increased metastatic capacity to the lungs of xenografted mice. In contrast, the ESR1-NOP2 fusion was sensitive to low estrogen conditions in vitro, and did not promote tumor growth. RNA-seq profiling revealed E2F targets pathway as the most highly enriched pathway induced by the ESR1 fusions. IHC revealed higher levels of pRb in ESR1-YAP1 and ESR1-PCDH11x xenograft tumors and subsequent CDK4/6 inhibition completely blocked tumor growth in an ESR1-YAP1 PDX model. Integrating RNA-seq with ChIP-seq data, we discovered a set of EMT and metastasis genes bound by all ESR1 fusions and WT-ER, but whose expression was strongly and uniquely up-regulated only by the ESR1-YAP1 and ESR1-PCDH11x fusions. These studies also revealed gained sites bound only by the ESR1-YAP1 and ESR1-PCDH11x fusions, not bound by WT-ER nor ESR1-NOP2. Genes mapping to these sites have a role in metastatic biology and were highly up-regulated by the YAP1 and PCDH11x fusions, potentially mediated by long range transcriptional activation.

**Conclusion.** ESR1-YAP1 and ESR1-PCDH11x are driver fusions that occur in drug-resistant, advanced stage breast cancer and are a new class of recurrent somatic mutation that can cause acquired endocrine therapy resistance, yet can be treated with CDK4/6 inhibition. These driver fusions also confer increased metastatic ability through their ability to drive expression of genes that contribute to EMT and metastasis. In contrast, ESR1-NOP2 did not produce functional protein and appears to be a passenger event. These studies may provide pre-clinical rationale for targeting ESR1 translocated breast tumors, since the presence of an ESR1 driver fusion places a patient in a therapeutic category where none of the currently available endocrine therapies are likely to be effective.
Evolutionary history and genomic landscape of metastatic breast cancer


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Body: Background: The majority of deaths from breast cancer are due to distant metastatic disease. Despite this, few systematic genomic analyses have been performed on metastatic tumors. This results from the relative difficulty of performing biopsies on metastatic tumors, as well as the uncertainty regarding genomic determinism, according to which the majority of actionable mutations present in metastases can be discovered in the primary tumor.

Methods: "METAMORPH" is an ongoing prospective cohort study of women with suspected or confirmed recurrent breast cancer enrolled prior to starting a new therapy for recurrent metastatic disease. Biopsies of metastatic lesions were performed under radiologic guidance, and archival primary tumors were subsequently obtained. WES and sWGS were performed to determine coding mutations and aberrant copy-number in metastatic tumors from 67 patients, 33 of which were assayed with corresponding matched primary tumors.

Results: Using Bayesian approaches, we find that cancers fit one of two patterns: canonical linear evolution (whereby the metastatic tumor arises from one or more advanced primary tumor subclones) vs. branched evolution (whereby both primary and metastatic tumors develop mutations that go on to become clonal within their respective tumors after the time of dissemination). In cases where tumors show evidence of branched evolution or small subclone dissemination, we expect that a large proportion of mutations may not be represented in both the primary and corresponding metastatic tumors. Indeed, primary-metastatic tumor pairs show substantial discordance at the genomic level, sharing only ~30% of mutations and ~28% of copy-number alterations on average. Furthermore, we find that metastatic tumors have decreased clonal heterogeneity, suggesting a history of selection. Indeed, we find clinically relevant mutations that are present exclusively in the primary or the corresponding recurrent metastatic tumor, as well as genes that are recurrently altered in metastatic tumors, such as amplification of SRC-1, loss of genes encoding CDK inhibitors, and alterations in JAK1/2/3. Finally, compared to the primary tumors from which they arose, metastatic tumors exhibit increased frequencies of alterations in several discrete pathways, including those involving the extracellular matrix as well as PI3K/AKT/mTOR, estrogen, and HER2 signaling.

Conclusions: The low degree of genomic concordance between primary and metastatic tumors due to evolutionary distance, as well as the presence of activating and targetable mutations specifically in metastatic tumors, suggests that there is value in comprehensively characterizing metastatic tumors to inform patient treatment and identify novel targets underlying breast cancer progression.
Body: Background
Invasive lobular carcinoma (ILC) is a common breast cancer histological subtype comprising ~10-15% of all cases. ILC possesses many unique features when compared to invasive ductal carcinoma (IDC). First, ILC has distinct genomic alterations expanding beyond the defining event of \( CDH1 \) loss to other genes such as \( TBX3, \) \( FOXA1, \) and AKT signaling related genes. Second, ILC responds differently to chemotherapeutics and endocrine therapies despite similar clinical staging. Third, ILC tumors spread to a distinct set of organs compared to IDC tumors, commonly forming distant metastases in the ovary, colon, omentum, and stomach. However, the genomics of metastatic ILC have yet to be fully explored.

Methods
Comprehensive hybrid-capture based genomic analysis of 286-395 cancer related genes was performed on 5523 histologically defined ILC \((n=613)\) and IDC \((n=4910)\) tumors. Of these, 29% and 21% were from distant metastatic sites for ILC and IDC, respectively. Additionally, histology based ER-status was available for a subset of tumors allowing a subgroup of ER-positive, HER2-negative IDC (ER-IDC) samples to be identified \((n=655)\).

Results
We examined the genetic differences between ILC and IDC in the context of both local and metastatic disease. Overall, the genomic profiles of ILC are enriched for alterations in \( CDH1, TBX3, PIK3CA, \) and \( RUNX1 \) in agreement with previous studies. Alterations in genes involved in AKT signaling \((PIK3CA, PTEN, \) and \( AKT1)\) are also enriched in ILC \((64\% v. 49\%; p<10^{-7})\). Interestingly, \( NF1 \) loss of function alterations are enriched in metastatic ILC compared to ER-IDC \((12.2\% v. 3.6\%, p<0.001)\) but not in local disease \((4.8\% v. 4.1\%, p=0.72)\). \( NF1 \) is a negative regulator of RAS-cyclic AMP pathway and suggests that \( NF1 \) driven RAS signaling is an important driver of metastasis in ILC.

We next examined metastatic ILC samples for alterations enriched at specific metastatic tissue sites. Two metastatic sites were exclusive to ILC samples compared to ER-IDC: GI \((19.4\%)\) and the female reproductive tract \((11.7\%)\). Within metastatic ILC, alterations in \( ESR1 \) showed strong tissue site bias towards liver metastases with 29% harboring an alteration in \( ESR1 \) \((\text{range: } 8-13\% \text{ in other sites, excluding ovary})\). Interestingly, \( ESR1 \) alterations were never observed in 14 ovary metastases, potentially reflecting an effect of local estrogen production on ILC ovarian metastases. In support of this, ILC ovarian metastases occur in younger women with a median age of 53.5 compared to 63.5 across all other sites.

Lastly, high tumor mutation burden (TMB) is strongly associated with metastatic ILC with 8.9% of metastatic ILC classified as TMB-high \((\geq 20 \text{ mutations/Mb})\) compared to 2.1% of ILC in the breast. A similar but less pronounced finding was also observed for ER-IDC \((1.6\% \text{ versus } 0.8\%)\). This suggests that checkpoint blockage therapies may be a more common option in metastatic ILC than previously appreciated.

Conclusions
Genomic profiling of metastatic ILC reveals numerous potential therapeutic options enriched in this disease. Inhibition of RAS signaling driven by \( NF1 \) loss and TMB-high directed immunotherapeutics may be potential therapeutic options for a substantial portion of metastatic ILC patients.
Title: Attitudes towards use of archived biospecimens among patients with cancer

Jeffrey Peppercorn\(^1\), Eric Campbell\(^1\), Julia Rabin\(^1\), Katharine Quain\(^1\), Fay Hlubocky\(^2\), Deborah Colyar\(^1\), Lecia Sequist\(^1\), Aditya Bardia\(^1\), Nora Horick\(^1\), Steve Isakoff\(^1\) and Deborah Mathews\(^2\). \(^1\)Massachusetts General Hospital, Boston, MA; \(^2\)Johns Hopkins, Baltimore, MD and \(^3\)University of Chicago, Chicago, Illinois.

Body: Background: Oncology research increasingly involves biospecimen collection and data-sharing. Ethical questions have emerged when researchers seek to use archived biospecimens for purposes that were not well defined in the original informed consent document (ICD). We sought to inform ongoing debates by assessing patient views on these issues.

Methods: We administered a cross-sectional self administered anonymous paper survey among patients at the Massachusetts General Hospital Cancer Center. Survey questions addressed attitudes towards cancer research and willingness to donate biospecimens, expectations regarding use of biospecimens and protections of research participants, and preferences regarding specific ethical dilemmas regarding use of archived biospecimens. Results are descriptive with comparisons among participants on the sociodemographic and clinical characteristics using chi-square and Fisher's exact tests.

Results: 187 patients offered participation agreed and returned the survey (Response rate 66%). Mean age was 59 (range 2 to 91), 81% were women, 86% were white, and 81% were college educated. Among all participants, 67% had breast cancer and 33% metastatic disease. 34% had participated in a clinical trial, 27% had donated tissue for research and 93% indicated willingness to donate tissue for research. The vast majority of participants (94%) expected both that donated tissue would be used to help as many patients as possible and (92%) that privacy of a donors health information would be carefully protected. 33% expected that donated tissue would only be used for research they specifically approved and 44% that data would not be shared with other researchers. We presented 3 hypothetical scenarios in which researchers sought to use stored biospecimens from a breast cancer clinical trial for future research that was not described in the original ICD. For scenario 1, in which the ICD stated tissue would only be used for breast cancer research, 75% supported use of tissue to study other cancers as well. For scenario 2, in which the ICD specified somatic genetic research only, 89% supported use of tissue for germline research if deemed important by investigators. For scenario 3, in which the ICD stated that data would not be shared beyond the investigators, 72% supported data sharing within a national data repository. Only 28% of participants endorsed concerns that a patient could be identified from their genetic information and 12% were concerned with potential harms from donation to biobanks. However, 38% felt that they owned their tissue and should control how it is used. We did not detect significant differences in responses on the basis of sociodemographic characteristics, cancer type, disease stage, or research experience.

Conclusion: Patients with cancer are highly supportive of tissue donation for research and expect that donated tissue will be used to maximize scientific results. They also expect that interests of research participants will be protected. When there is uncertainty regarding the use of archived biospecimens based on historical ICD and inability to recontact research participants, the interest of participants in seeing productive use of their tissue for science should be considered.
Identifying steroid hormone receptor gene mutations in patients with newly diagnosed estrogen receptor positive metastatic breast cancer

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Background: Resistance to endocrine therapy remains a challenge for patients with metastatic hormone receptor positive breast cancer. Mutations in the gene encoding estrogen receptor alpha (ESR1) have been identified in patients with endocrine resistance and correlates with reduced survival. The occurrence of mutations in the gene for progesterone receptor (PGR), another important biomarker in breast cancer, has not been reported.

Objective: Determine the frequency of mutations in ESR1 and PGR in patients with newly diagnosed metastatic ER+ breast cancer treated with adjuvant endocrine therapy at our institution, and identify whether these mutations are associated with worse patient survival.

Methods: This is an IRB-approved, HIPAA-compliant retrospective study of patients with ER+ metastatic breast cancer identified from our comprehensive cancer center registry. Eligible patients must have received at least 6 months of adjuvant endocrine therapy with biopsy-proven diagnosis of metastatic or locally recurrent disease. Next-generation sequencing of the coding regions of ESR1 and PGR was performed on extracted tumor genomic DNA. Analysis was limited to protein coding mutations. Clinicopathologic data was collected and correlated with the genomic information for each patient. Associations between mutation status and clinicopathologic factors were analyzed using Fisher’s exact test. Kaplan-Meier method with log-rank test was used to analyze overall survival (OS) from time of metastatic diagnosis.

Results: The study included 35 women (35-88 yr old). Metastatic sites included bone (N=13), brain (N=6), chest wall (N=2), liver (N=4), lung/pleura (N=4), and lymph nodes (N=6). PGR mutations were identified in 66% of patients (23/35; 95%CI 48-81%), and were more common than ESR1 mutations (10/35; 29%; 95%CI 15-46%). Mutations in both PGR and ESR1 were identified in 26% (9/35), and neither mutation was identified in 31% (11/35). Neither mutation was associated with prior adjuvant aromatase inhibitor or tamoxifen therapy. There was no significant association between ESR1 or PGR mutations and metastatic site, although 75% (3/4) of patients with liver metastases had an ESR1 mutation compared to 23% (7/31) without liver metastases (p=0.061). The differences in median OS between ESR1 wildtype vs mutant (1.6 mo vs 1.0 mo, p=0.14) and PGR wildtype vs mutant (2.6 mo vs 1.3 mo, p=0.56) were not significant. However, patients with both mutations may have worse median OS compared to patients with neither or only one mutation (1.0 mo vs 1.6 mo vs 4.0 mo, p = 0.073).

Conclusions: The overall survival of metastatic ER+ breast cancer patients with ESR1 or PGR mutations were not statistically significant compared with those without mutations. Despite the small sample size, there is a trend that patients with mutations in both genes may have worse survival. To the best of our knowledge, the PGR mutation rate in metastatic breast cancer has not previously been reported. Interestingly in our data set, PGR mutations were identified more commonly than mutations in ESR1. The significance of PGR mutations is not known, nor whether this could be a future indicator of treatment resistance or a target for therapy.
Title: ESR1 mutations confer novel metastatic functions in genome-edited breast cancer cell models

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Body: Background: Estrogen receptor alpha (ER\(\alpha\)), encoded by the ESR1 gene, is expressed in approximately 70% of breast cancers. Recent studies conducted by us and others have shown that somatic mutations in ESR1 gene play a key role in conferring endocrine resistance in ER\(\alpha\)+ breast cancer. These hotspot mutations mainly occur on the ligand binding domain of ER\(\alpha\), leading to poor outcomes in 25-30% of patients with ER\(\alpha\)+ metastatic breast cancer in clinic. The mechanisms behind the potential enhanced metastasis of these mutations have become an urgent issue to be addressed, but they are not well understood due to a lack of ESR1 mutant models.

Methods: We generated and characterized genome-edited T47D and MCF7 breast cancer cell lines with the two most common ESR1 mutations (Y537S and D538G), using CRIPSR/Cas9 and rAAV systems respectively. Multiple clones for each mutant were sorted and the mutation frequencies were detected using digital droplet PCR (ddPCR). We subsequently performed an RNA-sequencing to deeply differentiate the gene expression patterns in these mutants. The growth of these pooled mutant-cells was determined in both 2D and 3D plates. The cell-matrix adhesions were measured based on ECM array, and 84-ECM adhesion related genes were further tested by qPCR array. IncuCyte real-time image system and boyden chamber transwell assays were used to monitor the cell migration and chemotaxis. Tail vein injection were performed on nude mice, and immunofluorescent staining of lung tissues with human specific cytokeratin 19 were utilized to evaluate in vivo metastatic capacities of the mutant cell models.

Results: We first identified the robust mutation frequencies at both RNA and DNA levels in our cell models. The RNA-seq exhibits multiple ligand-independent genes overlapping between either cell lines or mutants, which were further conformed by qPCR. We also found that both Y537S and D538G mutants present ligand-independent growth in 2D and 3D ultra-low attachment plates. Using wound-scratching assay, we observed significant higher migration rate in D538G mutant of T47D cell lines on both matrigel and type I collagen, indicating a cell-line and mutant-specific phenotype. We also detected lower attachment of both mutants on type I collagen in both cell lines, and our qPCR array revealed that alterations in the MMP pathways could be one of the major mechanism causing this phenotype. Finally, tail vein injection of T47D mutant-cells in nude mice derived more micrometatsatic spots in the lung tissues.

Conclusion: In sum, our study presents the first in-depth metastatic functional analysis of the biology of ESR1 mutations in genomic knock-in cell models, pointing out the enhanced migration and decreased cell-matrix adhesion as a potential novel gain-of-function of the Y537S and D538G mutant-cells in vitro and in vivo. These findings suggest the potential role of enhanced metastasis of these ESR1 mutations through remodeling of transcriptional profiles, shedding lights towards the development of efficient therapies of ESR1 mutant breast cancer.
Title: Bi-allelic alterations in homologous recombination (HR) DNA repair-related genes as the basis for HR defects in human cancers: A pan-cancer genomics and functional analysis

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Body: Background: BRCA1 and BRCA2 are involved in homologous recombination (HR) DNA repair and are germ-line cancer pre-disposition genes that result in the hereditary breast and ovarian cancer (HBOC) syndrome. Whether germ-line or somatic alterations in these genes or other members of the HR pathway and if mono- or bi-allelic alterations of HR-related genes have a phenotypic impact in breast and other cancers remains to be fully elucidated. Here we took a combined genomic and functional approach to identify the role of mutations in HR-related genes and their impact on HR DNA repair.

Methods: Whole-exome sequencing and Affymetrix SNP6 array data from 8,178 tumors, comprising 24 different cancer types including breast cancer, were retrieved from The Cancer Genome Atlas (TCGA). We identified the prevalence of missense and pathogenic (frame-shift, nonsense, start/stop codon and splice site variants) somatic and germline mutations in 102 HR-related genes curated from the literature. For each mutation, we determined if the alterations were bi-allelic. We evaluated genomic signatures of HR-deficiency in each tumor using large-scale state transitions (LSTs) and a mutational signature of HR-deficiency (signature 3). An independent set of 24 fresh sporadic breast cancer tissue specimens from our institution was subjected to i) an ex-vivo assay that assesses the ability of cancer cells to form RAD51 foci in response to ex-vivo irradiation (IR), and ii) whole exome-sequencing to define whether RAD51 deficient tumors would display LSTs, signature 3 and bi-allelic inactivation of HR-related genes.

Results: 13% and 5% of all TCGA cases displayed pathogenic mono- and bi-allelic alterations of HR-related genes, respectively. Of the biallelic alterations, only 45% occurred in traditional BRCA1/2 associated hereditary cancers (HBOCs, namely breast, ovarian and prostate cancer). Bi-allelic, but not mono-allelic, pathogenic genetic alterations in HR-related genes were significantly associated with genomic evidence of HR deficiency across cancer types, in HBOCs and within breast cancer. In HBOCs, bi-allelic alterations in HR-related genes were mutually exclusive (p=0.02). In breast cancer, bi-allelic inactivation of HR DNA repair-related genes was observed in 9.8%, of which 7.8% involved a germline pathogenic mutation and 2.0% were solely somatic. In breast cancer, in addition to BRCA1 and BRCA2, bi-allelic inactivation of PALB2 (0.2%), ATM (1.1%) and POLQ (0.3%) were found to be associated with genomic features of HR deficiency. In the 24 additional breast cancers, 9 were classified by the functional ex-vivo RAD51 assay as HR-deficient, 8 of which displayed bi-allelic inactivation of one HR-related gene, whereas only 1 of the 15 HR-proficient breast cancers harbored bi-allelic inactivation of HR-related genes (p<0.001).

Conclusion: Bi-allelic germline and somatic alterations of HR-related genes in addition to BRCA1 and BRCA2 are present in breast and other cancer types. Irrespective of the gene, these bi-allelic alterations are associated with HR deficiency as defined by genomic methods and functional assays, expanding the potential opportunities for therapies targeting HR DNA repair defects.
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Title: APOBEC mutation signature in breast cancer correlates with tumor mutation burden and poor responses to therapy

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Body: Introduction
Mutational processes can be characterized by unique combinations of mutation types in the form of mutational signatures and have been associated with age, known mutagenic exposures, defects in DNA maintenance, or the APOBEC family of cytidine deaminases. We asked whether mutation signatures could be extracted from DNA sequence information in a targeted 434 gene panel covering 297 breast cancer specimens.

Materials and Methods
Targeted whole exome sequencing (Illumina, 2x50bp) of a 434 gene panel was performed on a set of 297 primary and metastatic breast tumor samples. Tissue of origin included breast (56%), liver (15%), lymph node (10%), lung (3%) and others (16%). Alignment was done with BWA against the human reference hg19 and variant calling was performed using VarDict. Germline variants were filtered based on allele frequencies, cohort specific population frequencies, as well as using 1000 Genomes and ExAC population frequencies. For somatic signature inference, only single nucleotide variants were retained. Panel specific trinucleotide frequencies were computed and normalized towards whole genome frequencies and somatic signatures were inferred using deconstructSigs method.

Results
We identified a total of 26 signatures from the set of 30 known signatures in our patient samples. Due to the small panel size, there was only a limited number of mutations available per patient to infer somatic signatures. On average, we identified two somatic signatures per sample. Most common mutation signatures identified were: Signature 1 (90.8%) - result of an endogenous mutational process initiated by spontaneous deamination of 5-methylcytosine; Signature 6 (21.8%) - defective DNA mismatch repair; Signature 15 (15.6%) - defective DNA mismatch repair; Signature 7 (9.9%) - ultraviolet light exposure; and Signature 10 (6.5%) - altered activity of POLE. An APOBEC specific signature was identified in 20 (7%) samples. APOBEC positive samples showed significantly higher tumor mutational burden (10.7 vs. 5.7 mutations/mb) as compared to APOBEC negative samples (p<=0.001). PIK3CA was found to be mutated in 80% of APOBEC positive samples, compared to 36% of APOBEC negative samples. In addition, we found higher rates of mutations in TP53 (70% vs. 50%), MLL3 (50% vs. 19%) and MLL2 (25% vs 14%) of APOBEC positive patients. Response rates of APOBEC positive patients were significantly worse than of APOBEC negative patients, with 50 percent of patients having progressive disease compared to 25 percent of APOBEC negative patients(p=0.07, borderline).

Conclusions
We demonstrate the feasibility of a targeted sequencing approach to extract somatic mutation signatures from breast tumor samples, and we highlight the potential of using the APOBEC signature to predict therapeutic responses.
Title: APOBEC3 contributes to mutational load in breast cancer

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Body: Breast cancer results in large part from the accumulation of multiple mutations in premalignant cells, which provide a molecular basis for genetic diversity. This genetic diversity in premalignant cells allows selection for increased proliferation and survival and ultimately leads to invasion, metastasis, and therapeutic resistance. Recent genome-wide sequencing data showed that APOBEC3B (A3B) contributes to mutational load in breast cancer. A3B, a DNA cytosine deaminase, is overexpressed in more than 50% of breast tumors and more than 75% of breast cancer cell lines. Its overexpression and aberrant activation lead to unexpected clusters of mutations in the majority of breast cancers. This phenomenon of clustered mutations, termed *kataegis* (*shower* in Greek) forms a unique mutation signature in breast cancer. On the basis of the finding that A3B is a key molecular determinant of the mutator phenotype in breast cancer, the goal of our research is to utilize informatics tools to systematically characterize genetic alterations of APOBEC3 family proteins in breast cancer genomic data and define the molecular impact of altered APOBEC3 family proteins on mutability and anti-tumor immunity.

Our data showed that the mutation rate and copy number amplification/deletion of APOBEC3 genes are low. The levels of APOBEC3A (A3A) and A3B are highly correlated and are highest in Basal subtype and lowest in Luminal A tumors, in concordance with the proliferation of subtypes. Additionally, A3A and A3B are significantly correlated with total mutational load as well as with TP53 mutation, and with somatic copy number alterations (SCNA), especially focal SCNA. Among APOBEC3 genes, A3B is significantly associated DNA replication, DNA damage repair, cell cycle and proteasome signatures, and shows predictive and prognostic capacity in ER-positive patients. Interestingly, A3G expression is strongly associated with immune response signature genes in all breast tumors. Consequently, A3G is highly associated with tumor-infiltrating lymphocytes in breast and several other disease types.

In summary, our data demonstrate distinct expression pattern of APOBEC3 genes in different breast cancer subpopulations. Overexpression of different APOBEC3 family members leads to distinct molecular consequences. These data provide new molecular insights into pathophysiological functions of APOBEC3 genes in breast cancer and provide therapeutic opportunities for the breast cancer patients whose tumors have altered APOBEC3 expression levels and potentially are driven by APOBEC3 genes. Importantly, APOBEC3G is associated with evidence of immune activation that may signal responsiveness to immune checkpoint inhibitors.
Title: Mutational processes, genome evolution and outcome in metastatic breast cancers

Body: Background: to determine the distribution and evolution of mutational processes in metastatic breast cancers (mBC), together with their clinical relevance. Methods: Whole exome sequencing (Hi-Seq, Illumina) and determination of copy number alterations (CNA) (CGH array / SNP6.0) were performed in 240 and 692 metastatic breast cancers respectively. Mutational processes were defined according to Alexandrov (Nature, 2013). Homologous Recombination Deficiency (HRD) was determined by genome wide assessment of loss-of-heterozygosity (LOH) on SNP6.0 (n = 210). Finally, genomic instability was assessed by the % of genome altered assessed by CGH / SNP6.0 Results: Whole exome sequencing showed that HR+/Her2- metastatic breast cancer presented an increased contribution of APOBEC-related signatures, as compared to early breast cancer (TCGA) (58% of the mutations vs 31%, p < 0.0001). Twelve percent of the HR+/Her2- mBC acquired an hypermutator genotype ( > 200 non-synonymous mutations). This acquisition of an hypermutator genotype was confirmed in five paired primary-metastatic samples. An operational APOBEC-related signature 13 was associated with a poor outcome in a multivariate analysis (HR: 1.75, 95%CI: 1.1-2.7, p = 0.017). High LOH score (HRD) was observed in 30% of HR+/Her2- mBC as compared to 13% of early HR+/Her2- early BC (p < 0.0001). The opposite was observed in TNBC (43% in mTNBC versus 58% in early TNBC, p = 0.032). High LOH score was associated with a trend for poor outcome in HR+/Her2- mBC (multivariate 1.67, 95%CI: 0.949-2.951, p = 0.075). The % of genome altered was associated with a poor outcome in multivariate analyses both in the overall and HR+/Her2- mBC (HR / 10 increase: 1.144, 95%CI:1.038-1.261, p = 0.007 and HR:1.18, 95%CI:1.037-1.344, p = 0.012 respectively). Copy number analyses identified 143 genes that are more frequently amplified as compared to early breast cancers (FDR < 0.01) Conclusions: metastatic HR+/Her2- metastatic breast cancer present an increased in APOBEC-related mutational burden and in LOH score as compared to early breast cancers. APOBEC-related signature 13 and genome instability are associated with a poor outcome and could be used in the future to better stratify metastatic breast cancer patients.
Title: IMpassion131: A phase III study comparing 1L atezolizumab with paclitaxel vs placebo with paclitaxel in treatment-naive patients with inoperable locally advanced or metastatic triple negative breast cancer (TNBC)

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Body: Background: Chemotherapy (including paclitaxel) remains the predominant treatment for metastatic TNBC but clinical outcomes remain poor. Therefore, new therapeutic approaches are needed. Atezolizumab blocks the binding of PD-L1 to its receptors PD-1 and B7.1, thus restoring tumor-specific T cell immunity. TNBC is a rational target for atezolizumab therapy due to high PD-L1 expression on tumor-infiltrating immune cells (IC) and elevated T cell tumor infiltration. Furthermore, combining chemotherapy with atezolizumab is hypothesized to enhance anti-tumor immune response via neoantigen release. Atezolizumab alone and in combination with nab-paclitaxel has demonstrated promising clinical benefit in metastatic TNBC and was well tolerated, with no exacerbation of chemotherapy-associated adverse events. Atezolizumab in combination with nab-paclitaxel is being further investigated as 1L TNBC treatment in IMpassion130. IMpassion131 is a global, multi-center, randomized, double-blind, placebo-controlled study comparing the efficacy and safety of 1L atezolizumab + paclitaxel vs placebo + paclitaxel in patients with untreated, inoperable, locally advanced or metastatic TNBC. (NCT03125902)

Methods: Eligibility criteria include patients with inoperable, locally advanced or metastatic TNBC, histologically confirmed; de novo or recurrent disease after early breast cancer treated with chemotherapy ≥ 12 months prior; eligible for taxane monotherapy; no prior chemotherapy or targeted systemic therapy for inoperable locally advanced or metastatic disease; ECOG PS 0-1 and measurable disease by RECIST v1.1. Exclusion criteria include known symptomatic CNS disease, prior immunotherapy and a history of autoimmune disease. Approximately 495 patients will be randomized 2:1 to receive atezolizumab (840 mg) or placebo (q2w; days 1 and 15 of 28-day cycle) plus paclitaxel (90 mg/m²; days 1, 8, 15 of 28-day cycle) until disease progression. Stratification factors are PD-L1 expression on tumor-infiltrating IC (IC0 < 1% vs IC1/2/3 ≥ 1% with VENTANA SP142 IHC assay), prior taxane therapy, presence of liver metastases and geographical region. The primary endpoint is progression-free survival (PFS) measured by RECIST v1.1. Key secondary endpoints include overall survival (OS), 12- and 18-month OS rates, 12-month PFS rate, objective response rate, duration of response, and safety. Tumor biopsies will be collected at baseline, on treatment and at disease progression to assess for biomarkers of treatment response and immune escape.
Title: A multicenter phase II trial to evaluate the efficacy and safety of pembrolizumab and gemcitabine in patients with HER2-negative advanced breast cancer: GEICAM/2015-04 PANGEA-Breast

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Body: Background: Treatment options for advanced breast cancer (ABC) are multiple but unable to properly respond to current clinical needs. In particular, improved therapies are needed for triple negative and hormone receptor (HR)-positive but heavily pretreated patients. Pembrolizumab (P) is a human monoclonal antibody that blocks the PD-1/PD-L1 interaction hence potentiates anticancer T cell responses. Gemcitabine (G) is a cytotoxic drug with well-known immunostimulatory properties. Here, we report an ongoing phase II clinical trial to identify the Recommended Phase II Dose (RP2D) and the efficacy of the combination of these two agents in ABC patients. We hypothesize that these agents may synergize to induce responses with long term clinical benefit (ClinicalTrials.gov Identifier: NCT03025880).

Trial Design: Eligible patients are HER2-negative ABC patients who received prior treatment with anthracyclines and taxanes and two or more prior lines of hormone therapy, if HR-positive disease. Patients with CNS involvement are also eligible if clinically stable. Treatment consists of 21-day cycles with 200 mg P on day 1 and G on days 1 and 8. In the safety dose testing, we use a standard 6+6 design with 2 dose levels (DL) of G: 1250 mg/m² (DL0) and 1000 mg/m² (DL1). Patients are treated until radiologic or symptomatic progression, or unacceptable toxicity. The primary objectives are RP2D and objective response rate (ORR) of the combination; secondary objectives include evaluation of safety and tolerability and other efficacy variables (progression-free survival [PFS], clinical benefit rate [CBR], response duration [RD] and overall survival [OS]). Efficacy is measured by RECIST 1.1. and irRECIST. Safety is measured using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) 4.0. As exploratory objectives, immunological biomarkers are analyzed in tumor biopsies and blood samples and correlated with (1) clinical efficacy and (2) disease outcomes. Sequential tumor samples are collected at baseline, cycle 3 and at progression. Blood samples are drawn at baseline, cycle 3, and cycle 6, or at post-treatment visit (whatever occurs first). Tumor samples are characterized for intratumoral and stromal tumor-infiltrating lymphocytes, tumor-associated macrophages and myeloid-derived suppressor cells, PD-L1 expression in tumor cells and stroma. Moreover, molecular and genetic profiling will be performed. Blood samples are characterized for peripheral blood mononuclear cell (PBMC) phenotype (including expression of co-activatory and co-inhibitory receptors), cytokine profile, and activity of other immunosuppressive pathways (e.g., IDO1-dependent tryptophan catabolism). These results will be compared with data from a cohort of healthy volunteers. A maximum of 65 patients will be included. The study is approved by the ethical committee and Competent Authority of Spain and already open for patient recruitment in 2 of the 10 participating sites.

Keywords: Breast
HER2 negative
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Title: A phase II multi-center study of BGB324 in combination with pembrolizumab in patients with previously treated, locally advanced and unresectable or metastatic triple negative breast cancer (TNBC) or triple negative inflammatory breast cancer (TN-IBC)

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Body: Background. The AXL receptor tyrosine kinase is associated with poor overall survival in breast cancer. AXL signaling is an important regulator of tumor plasticity related to epithelial-to-mesenchymal transition (EMT) and stem cell traits that drive metastasis and drug resistance. Upregulation of AXL has been associated with reduced response to anti-PD-1 therapy. Signaling via AXL is also a key suppressor of the anti-tumor innate immune response, and AXL is expressed on several cells associated with the tumor immune microenvironment. Hence AXL signaling contributes uniquely to both tumor cell intrinsic and microenvironmental anti-tumor immune suppression mechanisms. We show that AXL is required for tumor immune evasion in the 4T1/Balb/C mammary adenocarcinoma model and that blocking AXL signaling with BGB324, a selective clinical-stage small molecule AXL kinase inhibitor, enhanced the effect of immune checkpoint blockade. BGB324 + anti-CTLA-4/anti-PD-1 treated tumors displayed enhanced infiltration of cytotoxic T lymphocytes and Natural Killer cells. Importantly, responding animals rejected orthotopic 4T1 tumor cell re-challenge, demonstrating sustained tumor immunity. These data provided a translational rationale for combining AXL targeted therapy with immune checkpoint inhibitors to enhance anti-cancer immune response.

Study Design. BGBC007 (NCT03184558) is an open-label, single arm, multi-center phase II study designed to assess the anti-tumor activity of BGB324 in combination with pembrolizumab in patients with previously treated, locally advanced and unresectable, or metastatic TNBC or TN-IBC. Secondary objectives include safety and pharmacokinetic profile of BGB324 and pembrolizumab in combination. A single arm, extension of Simon’s 2-stage design is employed with an interim and final analysis. Up to 56 evaluable patients will be enrolled. Recruitment will be halted once 28 evaluable patients have been entered to determine the Objective Response Rate (ORR, complete response and partial response). If 5 or fewer responses are observed in up to 28 patients, the trial will be terminated in favor of the null for futility. If 11 or more responses are observed, then the trial will be stopped in favor of the alternative for demonstration of activity. If 6 to 10 patients have an observed response then a further 28 patients may be evaluated. This design provides an overall power of 80.6% to test the stated null and alternative hypothesis.

BGB324 will be administered orally, once daily, in a fasted state. Days 1, 2 and 3 of BGB324 administration consists of a ‘loading’ dose of 400 mg followed by a dose of 200 mg daily. A fixed dose of 200 mg pembrolizumab will be given by intravenous infusion over 30 minutes every 3 weeks. BGB324 and pembrolizumab will be given until disease progression, unacceptable dose toxicity, or until 106 weeks (35 cycles). Efficacy endpoints including ORR, Duration of Response, Progression Free Survival are based on tumor imaging evaluation by RECIST 1.1. Tumor specimens will be taken to assess AXL and PD-L1 expression.
Title: A phase 2, open-label study of imprime PGG (Imprime), a novel beta glucan, with pembrolizumab (Pembro) in chemotherapy-resistant metastatic triple negative breast cancer (TNBC)

Body: By blocking the interaction of PD-L1 with PD-1, immune checkpoint inhibitors (CPI) can unleash specific, anti-cancer killing function of activated cytotoxic T cells in patients (pts) for whom there is evidence of an ongoing anti-cancer immune response (PD-L1 expression and/or activated T cells within the tumor bed). Single agent CPI therapy has provided substantial clinical benefit to pts with multiple cancer types. Though effective, response rates are typically limited (~15-30% of pts depending on tumor type) and therapy fails to benefit the majority of pts. For these pts there is often limited or no evidence of an ongoing T cell-based immune response. Agents that stimulate the anti-cancer immune response may be particularly promising in expanding the clinical responsiveness to CPI therapies. Imprime is a novel beta glucan derived from Saccharomyces acting mechanistically as a pathogen-associated molecular pattern (PAMP) or non-self danger signal, to awaken and activate the innate immune system. Imprime drives a cascade of immune activating events activating tumor-specific cytotoxic T cells. Imprime treatment elicits repolarization of the immunosuppressive microenvironment while activating the maturation of antigen presenting cells. Imprime has significantly enhanced the efficacy of CPI therapy in preclinical tumor models. In humans, Imprime-mediated innate immune activation requires the formation of an immune complex with naturally-occurring anti-beta glucan antibodies (ABA). Formation of this complex is dependent upon sufficient ABA levels. Imprime is now being studied in combination with pembro (KEYTRUDA®), a humanized mAb against PD-1 which has been previously studied in TNBC pts. This phase 2 study explores the treatment combination in pts with metastatic TNBC who progressed following at least one line of chemotherapy and pts with metastatic melanoma who progressed following CPI therapy with sufficient pre-treatment ABA levels (~50% of screened patients). The study is a Simon 2-stage design. Specific to the TNBC tumor type, a sample size of 12 pts in Stage 1 is planned. Criteria to advance to Stage 2 are ≤4 GR 3/4 AEs and ≥2 objective responses in TNBC. An additional 30 TNBC pts may be enrolled in Stage 2. Main eligibility criteria are metastatic TNBC after chemotherapy in the metastatic setting and serum ABA ≥20 µg/mL. The primary endpoints are ORR and safety; secondary endpoints are TTR, CRR, DoR, PFS, and OS. Efficacy will be analyzed for ORR and CRR as point estimates with 95% CI and for PFS, OS, DoR and TTR as descriptive summaries. Safety parameters will be summarized. Exploratory endpoints include ORR and PFS based on irRECIST. This study aims to collect pre- and early on-treatment tumor (6 wks post-1st dose) biopsies and peripheral blood to assess the impact of the treatment combination on immune activating events in the periphery and at the tumor site. As of June 2017, 11 sites were open and 4 pts were in treatment. The trial is sponsored by Biothera Pharmaceuticals, Inc. in collaboration with Merck & Co. (ClinicalTrials.gov NCT02981303) For information, contact Richard D. Huhn, MD, Biothera Med Dir at rhuhn@biothera.com or 651-256-4657.
Title: A phase II study using talimogene laherparepvec as a single agent for inflammatory breast cancer or non-inflammatory breast cancer patients with inoperable local recurrence

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Body: Objective: The primary purpose of the study is to determine the local and systemic antitumor efficacy of talimogene laherparepvec in locally recurrent breast cancer patients with or without distant metastases, as evidenced by improved overall response rates. This will be the first study to use biopsy of distant disease to demonstrate whether systemic immune modulation has antitumor efficacy in breast cancer patients.

BACKGROUND: Patients with locally recurrent breast disease frequently undergo multimodal treatment at the first occurrence of breast cancer, and because local treatment modalities such as surgical intervention and radiation are difficult to add, they subsequently receive systemic therapy. Talimogene laherparepvec (T-VEC) was developed to eliminate solid tumors and has since been considered as a potential treatment option for body surface tumors. In addition to T-VEC injected area, this agent is capable of modifying the immune response with the potential of inhibiting distant metastases. Hence, locally recurrent breast disease could benefit from T-VEC regardless of concomitant distant metastases, and may offer a new local treatment option.

Study Design and Treatment Plan: This is a single agent phase II study. Patients with breast cancer who have recurrence of chest wall disease with or without distant metastasis, have at least 1 injectable lesion ≥5 mm in longest diameter or multiple injectable lesions that in aggregate have a longest diameter of ≥ 5 mm, and meet inclusion and exclusion criteria will be eligible to participate in the study. Patient will receive T-VEC via intra-tumoral injection every 2 weeks after the first initial injection (3 weeks).

STATISTICAL METHODS:
Up to 35 patients will be enrolled in the study. The trial will be conducted using a two-stage design and the overall response rate will be estimated accordingly. It is assumed that the talimogene laherparepvec single agent will have a response rate of 20%. A response rate of 5% or lower will be considered treatment failure and the regimen will be rejected under this circumstance.

Status of the study:
Activation Date: Aug 2016. 6 patients have been treated. Enrollment continues.

Sponsor: Amgen
State of Texas appropriation for rare and aggressive breast cancer research.
2017 San Antonio Breast Cancer Symposium

Publication Number: OT1-02-01

Title: A phase II study of anti-PD-1 (MK-3475) therapy in patients with metastatic inflammatory breast cancer (MIBC) or non-IBC triple negative breast cancer (non-IBC TNBC) who have achieved clinical response or stable disease to prior chemotherapy

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Body: Primary Objective: To assess the efficacy of MK-3475 as a single agent in patients with MIBC and non-IBC TNBC. The primary endpoint is disease control rate at the end of 4 months after receiving the treatment. We will also investigate the association between biomarkers in the peripheral blood and tumor tissue, safety and efficacy.

Background: The extensive invasion of lymphatic vessels by tumor emboli in patients with IBC suggests that the host immune surveillance system is suboptimal or that the tumor cells have decreased immunogenicity through immune editing to avoid detection by the host. In the immune-competent host, tumor cells must overcome both innate and adaptive immunologic defenses of the host. The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. MK-3475 is a potent and highly selective humanized mAb designed to block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. MK-3475 strongly enhances T lymphocyte immune responses in cultured blood cells from healthy human donors, cancer patients, and primates. Mouse anti-PD-1, as a monotherapy, demonstrated efficacy in several syngeneic mouse tumor models. To date, no specific targeted therapeutic options exist for the treatment of MIBC and TNBC. After patients achieving a clinical response to systemic therapy, the maintenance of disease control is not guaranteed. Further, our recent publication suggests that IBC has immune dysfunction. Chemotherapies can debulk the disease volume but cannot be used for maintenance due to their toxicities. Using an anti PD-1 monoclonal antibody is a promising approach for this patient population.

Study Design and Treatment Plan: This is a single arm phase II study. Up to 35 patients with HER2 negative MIBC or metastatic TN-IBC (MTNBC) who have achieved clinical response or stable disease after receiving any prior systemic therapy for metastatic/recurrent disease, and meet all other criteria will be eligible. Patients will receive MK-3475 200 mg IV every 3 weeks for up to 2 years.

Statistical Considerations: The trial will be conducted using Simon's optimal two-stage design and the rate of disease control will be estimated accordingly. It is assumed that the MK-3475 single agent will have a disease control rate of 30%. A disease control rate of 10% or lower will be considered treatment failure and the regimen will be rejected under this circumstance.

Status of the study:
Activation Date: June 2015. 13 patients have been enrolled. Enrollment continues.

Sponsor: Merck Sharp & Dohme Corp.
State of Texas appropriation for rare and aggressive breast cancer research.
2017 San Antonio Breast Cancer Symposium

Publication Number: OT1-02-02

Title: A phase II study of pembrolizumab in combination with palliative radiotherapy for metastatic hormone receptor positive breast cancer

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Body: BACKGROUND: Despite recent advances in the treatment of patients with metastatic hormone receptor positive (HR+)/HER2- breast cancer (BC), it remains an incurable disease. The activity of immune checkpoint inhibitors (ICI) as monotherapy in patients with metastatic HR+/HER2- BC has been limited. Therefore, the addition of other strategies that elicit an immunogenic tumor microenvironment may be needed. We hypothesize that radiation therapy (RT) will potentiate the efficacy of the PD-1 inhibitor pembrolizumab in patients with metastatic HR+/HER2- BC. METHODS: Trial Design: This is a phase II single arm study assessing objective response rate (ORR) according to RECIST 1.1 in patients with metastatic HR+/HER2- BC who will receive pembrolizumab in combination with palliative RT. Pembrolizumab 200 mg intravenously will be administered 2-7 days before day 1 of RT, and will be given every 21 days until disease progression. Biopsies will be performed in the same lesion at baseline (mandatory if tumor tissue is accessible outside the field of RT) and during cycle 2 within 7-14 days before the day 1 of cycle 3 of pembrolizumab. Key Eligibility Criteria: Patients with metastatic HR+/HER2- BC, with measurable disease outside the field of radiation, for whom palliative RT to at least one bone, lymph node, or soft tissue lesion is indicated. Radiation of visceral lesions (such as lung or hepatic lesions) is not permitted. Although prior RT is allowed, patients must be at least 3 months free from RT; Re-irradiation of the same field is not allowed. There is no limit to the number of previous treatments, and systemic treatment naive patients for metastatic disease are also eligible. Specific Aims: The primary aim is to evaluate the efficacy of the combination, as defined by objective response rate (ORR) outside the field of RT according to RECIST 1.1. Secondary objectives include to determine the ORR according to immune-related criteria, the progression-free survival, the abscopal response rate, the clinical benefit rate, the safety and the tolerability of the combination. In addition, correlative studies will be performed to explore the correlation of immunosuppressive and/or immune-stimulating immune marker profiles at baseline and after cycle 2 to disease response to therapy. Statistical Methods: Using the Simons "optimal" method, in the first stage, 8 patients will be enrolled. If there is at least 1 response, accrual will continue to the second stage where up to 19 additional patients will be enrolled. If at least 3 of these 27 patients have an objective response (≥10%), the regimen will be considered worthy of further study. With this design, the probability of stopping the trial early is 78% if the true response rate is 3%. If the true response rate is 20% the chance that the regimen is declared worthy of further study is 80%. Patient accrual and target accrual: The trial opened in April/2017, and so far, has accrued 2 patients with a target accrual of 27 patients. Accrual should be complete in 14-25 months. Clinical trial information: NCT03051672.
2017 San Antonio Breast Cancer Symposium

Publication Number: OT1-02-03

Title: TBCRC 044: A randomized phase II study of pembrolizumab in combination with carboplatin versus carboplatin alone in breast cancer patients with chest wall disease

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Body: Background: Patients with breast cancer (BC) and chest wall disease have limited treatment options. We hypothesize that checkpoint inhibition may be an effective treatment approach due to the inflammatory nature of chest wall infiltration, and the association of PD-1 expression with lymphocytic infiltration. Platinum chemotherapy may facilitate anti-tumor immunity in a synergistic manner, and clinical studies of the PD-1 inhibitor pembrolizumab with platinum combinations have been effective in the treatment of advanced lung cancer. In this study, we will evaluate the combination of carboplatin and pembrolizumab in BC patients with chest wall disease.

Methods: This is a randomized phase II multicenter study in the TBCRC including patients with advanced, unresectable BC with hormone resistant or triple negative chest wall disease. Patients may have had prior surgery, prior chest wall radiation is not required, and other sites of distant metastases are allowed. Eighty-four patients at TBCRC sites will be randomized 2:1 to receive pembrolizumab and carboplatin (n=56, Arm A) or carboplatin alone (n=28, Arm B) until disease progression. Patients randomized to Arm A may cross-over following progression to pembrolizumab alone (Arm Bx). Patients in Arm A will be treated with pembrolizumab 200 mg IV and carboplatin AUC 5 IV every 3 weeks for at least 6 cycles followed by maintenance pembrolizumab 200 mg IV every 3 weeks until stable or responding disease. Patients in Arm B will be treated with carboplatin AUC 5 IV every 3 weeks until progression, then may cross-over to pembrolizumab 200 mg IV every 3 weeks alone (Arm Bx). An interim analysis for futility will be performed after 18 patients are enrolled into Arm B to allow early closure of that arm for lack of efficacy. The primary endpoint is disease control rate at 18 weeks of treatment; the study is powered to detect a 20% difference in disease control rates between arms (hazard ratio 0.52, $\alpha = 0.10$, $\beta = 0.20$). Secondary endpoints include progression free survival, toxicity, and response based on PD-L1 expression and irRECIST. Exploratory endpoints include association of response with a number of biomarkers including tumor PD-L1 gene expression, tumor and peripheral blood immune composition and cytokine expression, peripheral T-cell PD-1 expression, circulating tumor DNA, circulating tumor cells, and tumor MYC genomic expression using tumor biopsy and peripheral blood testing before and after treatment. This study should be open to accrual by August of 2017. (NCT03095352)
2017 San Antonio Breast Cancer Symposium

Publication Number: OT1-02-04

Title: SWOG S1418/NRG -BR006: A randomized, phase III trial to evaluate the efficacy and safety of MK-3475 as adjuvant therapy for triple receptor-negative breast cancer with ≥ 1 cm residual invasive cancer or positive lymph nodes (>pN1mic) after neoadjuvant chemotherapy

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Body: Background: Patients with residual cancer after neoadjuvant chemotherapy, particularly triple negative cancers (TNBC), have poor prognosis. The SWOG S1418 / NRG BR-006 (NCT02954874) randomized, phase III trial tests the hypothesis that administration of pembrolizumab after surgery for 12 months will reduce invasive disease-free survival (IDFS) by 33% compared to observation in patients with TNBC and ≥ 1 cm residual invasive cancer or positive lymph nodes (>pN1mic) after neoadjuvant chemotherapy.

Methods: Eligible patients ≥18 years old with triple negative breast cancer defined by ASCO/CAP guidelines and ≥ 1 cm residual invasive cancer in the breast, or any macrometastases in the lymph nodes after completion of 16-24 weeks of neoadjuvant chemotherapy. Patients may receive post-operative chemotherapy for up to 24 weeks but must be registered for screening within 35 days of completion of adjuvant chemo. Completion of radiation therapy prior to registration is allowed, but it is preferred that patients receive radiation after randomization; patients randomized to pembrolizumab will receive their XRT concomitant with pembrolizumab. Adequate organ functions: ANC > 1.5, PLT > 100, Hgb > 9, normal creatinine, Tbili < 1.5 IUNL, AST/ALT/AlkPhos ≤ 2.5 IULN. HIV with good CD4 count is allowed. Active autoimmune disease, Hep B,C, prior immunotherapy, active immunosuppressive therapy, or live vaccines within 30 days of registration are not allowed. Five unstained slides for PDL1 staining are required for stratification. The study has a dual primary endpoint; comparison of IDFS between arms in (i) all randomized patients (1-sided a=0.01) and in PDL-1 positive patients (1-sided a=0.015). Secondary endpoints include toxicity, overall survival, distant recurrence free survival (DRFS) and quality of life measures. Patients will be randomized 1:1 with stratification for PDL1 status, T size, nodal status and adjuvant chemo (yes or no) to observation or 1 year of pembrolizumab 200mg IV q 3 weeks. The accrual goal is N=1000 patients with estimated trial duration of 8 years. Two interim analyses are planned for all randomized patients when 50% and 75% of IDFS events have occurred for early stopping for either futility or efficacy. The study was activated on 11/15/16 and 34 patients were registered as of June 9, 2017. Cancer Trials Support Unit (CTSU) sites can use “OPEN” (https://open.ctsu.org) to enroll patients to this trial.

Funding: NIH/NCI U10CA180888, U10CA180819, CA180868; and in part by Merck, Sharpe & Dohme, Corporation.
Title: A single arm phase II study of adjuvant anti-PD1 (pembrolizumab) in combination with hormonal therapy in patients with hormone receptor (HR)-positive localized inflammatory breast cancer (IBC) who did not achieve a pathological complete response (pCR) to neoadjuvant chemotherapy

Angela Alexander¹, Jie Willey¹, Huiming Sun¹, Charla A Parker¹, Angela N Marx¹, Anita L Wood¹, Sangeetha M Reddy¹, James M Reuben¹, Roland L Bassett¹, Huong T Le-Petross¹, Savitri Krishnamurthy¹, Yun Gong¹, Wendy A Woodward¹, Vicente Valero¹, Naoto T Ueno¹ and Bora Lim¹. 'University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Background: The pCR rate to conventional chemotherapy in hormone receptor positive IBC has historically been low (7.4% for HR+ HER2-, and 30% for HR+ HER2+), and despite the use of adjuvant endocrine therapy, the recurrence rate is still as high as 40%. To date, no targeted agent is proven to improve the efficacy of adjuvant endocrine therapy within the IBC population to improve this poor disease free survival (DFS). One plausible reason for the poor efficacy of endocrine therapy is a suppressed immune system, which allows tumor cells to avoid detection despite expression of potential immunogenic surface antigens.

Trial Design: This is a single arm trial that will enroll stage III HR+ IBC patients who have completed neoadjuvant therapy but had residual disease at mastectomy. Enrollment should be before or within 2 months of beginning endocrine therapy. Monitoring of DFS will be done with radiological imaging every 3 cycles (starting at cycle 4) as clinically indicated, per standard of care. Pembrolizumab is given on day 1 of each 21 day cycle for up to 2 years if the disease is controlled, and hormonal therapy will be administered per standard of care.

Eligibility Criteria: Clinical stage 3 IBC ER+/PR+ and HER2 negative patients who completed neoadjuvant chemotherapy and surgery with evidence of residual cancer in the breast or lymph nodes, but be clinically disease-free with good performance status at the start of study. Patients also must have adequate hematologic and organ function, and have recovered from the acute effects from prior treatments.

Specific Aims: The primary objective is to determine the disease free survival (DFS) at 2 years of patients with adjuvant therapy using Pembrolizumab in combination with standard adjuvant hormonal therapy. The secondary objective is to determine the safety and toxicity profile of this combination.

Statistical Methods: With a sample size of 37 patients, assuming that 80% are alive (20% increase from historical data) and disease-free at 2 years, and all patients are followed for >2 years after enrollment with no dropout, a 95% confidence interval around the 2-year estimate of DFS will be generated. DFS will then be compared with the historical control rate of 60% by year 2 using a one-sided exponential MLE test.

Accrual: To date we have enrolled 3 patients since activation in January 2017, and the target enrollment is 37 patients.

Contact information: For more information or to refer a patient, please contact study coordinator, Angela Alexander - aalexand@mdanderson.org
2017 San Antonio Breast Cancer Symposium

Publication Number: OT1-03-01

Title: Chemotherapy-free trastuzumab and pertuzumab in HER2 [+] breast cancer: FDG-PET response-adapted strategy. The PHERGain study

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Body: BACKGROUND:
Several studies have confirmed that a significant subset of patients (pts) with early stage HER2[+]breast cancer (BC) achieve pathological complete response (pCR) with a dual HER2 neoadjuvant blockade without chemotherapy (chemo). Early metabolic evaluation using ¹⁸F-fluorodeoxyglucose (FDG) positron emission tomography/computed tomography (PET/CT) might help to recognize those pts with a higher likelihood of obtaining a pCR and an excellent outcome with a chemo-free strategy.

TRIAL DESIGN:
This is a randomized, multicenter, non-comparative phase II trial. Pts age ≥ 18 years with centrally-confirmed, treatment-naïve, HER2 [+] operable BC will be randomized, in a 1:4 ratio, and stratified by HR status, to receive docetaxel, carboplatin, trastuzumab, and pertuzumab (TCHP) (cohort A), or trastuzumab and pertuzumab (HP) ± endocrine therapy (ET) according to HR status (cohort B). Centrally-reviewed ¹⁸F-FDG PET/CT scans will be performed prior to randomization and after 2 cycles of therapy. Pts allocated into cohort A will continue with the same therapy for a total of 6 cycles regardless of ¹⁸F-FDG PET/CT results. Pts enrolled into cohort B showing at least a 40% reduction of the SUVmax on ¹⁸F-FDG PET/CT respect to baseline (PET responders) will continue with the same therapy for a total of 8 cycles. PET non-responders pts will receive 6 cycles of TCHP. After surgery, cohort B/PET responders pts who do not achieve a pCR will receive 6 cycles of TCHP. Moreover, all pts from cohorts A/B must complete 18 cycles of HP, along with adjuvant ET and radiotherapy (RT) according to HR status and institutional practices, respectively. Pts with subclinic metastases will be assigned to cohort C to receive 6 cycles of TCHP. Surgery and RT will be evaluated on a case-by-case basis on cohort C, and all pts will continue with HP for at least 12 additional cycles ± ET according to HR status.

The first co-primary endpoint is to evaluate the rate of pCR defined as the absence of invasive disease in the breast and axilla (ypT0/isN0) achieved with HP ± ET in PET responders pts (cohort B/PET responders). The second co-primary endpoint is to evaluate 3-year (3-y) invasive disease-free survival (iDFS) rate defined as time from the first date of no disease to invasive recurrence, new invasive disease, or death by any cause in cohort B.

Total accrual will be 400 pts. Considering a 10% and 25% of drop-out rates at the time of first and second co-primary analysis, the study will be positive if ≥41 pts achieved a pCR in cohort B/PET responders; or if we observe ≤14 events of 3-y iDFS in cohort B. Decisions will be based on one-sided, exact binomial test. With a 2.5% type I error rate (H0: pCR ≤20% and 3-y iDFS ≤89%) and 80% power (HA: pCR ≥30% and 3-y iDFS ≥95%).

The secondary objectives are to evaluate other definitions of pCR, rates of breast-conserving surgery, tumor response by magnetic resonance imaging, optimal ¹⁸F-FDG PET cut-off for pCR and other ¹⁸F-FDG PET quantification parameters for pCR prediction, DFS, distant-DFS, overall survival, progression-free survival, and health-related quality of life. Translational sub-studies will analyze biomarkers that may be predictive of response to dual HER2 blockade with HP.
Title: MP0274-CP101: A phase 1, first-in-human, single-arm, multi-center, open-label, dose escalation study to assess safety, tolerability, and pharmacokinetics of MP0274 in patients with advanced HER2-positive solid tumors

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Body: Background: MP0274 is a four domain, trispecific, biparatopic, HER2-targeted Designed Ankyrin Repeat Protein (DARPin®) drug candidate. M0274 binds to domains II and IV of HER2 at different sites from trastuzumab and pertuzumab, and to human serum albumin for an extended half-life. MP0274 displays a unique mode of action in preclinical models by directly inducing apoptosis in HER2-addicted cancer cells. Compared to anti-HER2 monoclonal antibodies, preclinical studies revealed no additional safety signals and no maximum tolerated dose was reached.

Trial Design: This is a first-in-human study with 2 parts. The dose escalation part of the study, follows a classical 3+3 dose escalation approach, including one extra cycle for exploration of specific PK characteristics, and will establish the recommended dose (RD) for further development based on safety, pharmacokinetics (PK), and preliminary efficacy. The extension part is designed to confirm safety and further estimate efficacy at the RD in additional patients. MP0274 monotherapy will be administered as infusion over one hour every 3 weeks until disease progression.

Eligibility Criteria: The study population for this first-in-human study is HER2-positive cancer patients who have progressed after standard therapy for advanced disease. Inclusion/exclusion criteria are similar to those in studies with anti-HER2 antibodies.

Specific Aims: The primary objective is to assess safety and tolerability. Secondary objectives are PK, preliminary anti-tumor efficacy (RECIST), and characterization of immunogenicity. Biomarker evaluation include exploratory markers such as PIK3CA, BCL2, p53, PTEN and p21 in both tissue or plasma (ctDNA), investigation of drug induced apoptotic markers as well as additional translational research. This may help get a deeper insight into MP0274’s mode of action and into potential resistance mechanisms. MP0274 may have the potential to be active against all HER2-dependent tumors including those that are resistant to trastuzumab and pertuzumab due to incomplete signaling inhibition or inadequate ADCC functionality. MP0274 could be a valid treatment option for HER2 positive cancers.

Statistical Methods: Due to being of non-comparative nature, no inferential statistical analysis will be applied in either the escalation or the expansion part of this study. Results will be listed and summarized by dose regimen using descriptive statistics. An interim evaluation of safety, tolerability and preliminary anti-tumor activity will be performed after 14 patients have been dosed at RD at least once and have completed end of Cycle 4. An interim analysis will be performed once all planned 29 patients have been dosed at RD at least once and completed end of Cycle 7 (pre-Cycle 8) tumor assessment. The final statistical analysis will occur after all patients have discontinued treatment for any reason and completed safety follow-up.

Present Accrual and target accrual: Currently recruitment is planned in UK, Germany, and Switzerland. Target accrual is 36 evaluable patients.
2017 San Antonio Breast Cancer Symposium

Publication Number: OT1-03-03

Title: Phase II, open label, randomized, biomarker study of immune-mediated mechanism of action of neoadjuvant subcutaneous trastuzumab in patients with operable or locally advanced/Inflammatory HER2-positive breast cancer. ImmunHER trial on behalf of the Gruppo Oncologico Italiano di Ricerca Clinica (GOIRC)

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Body: Background: Tumor-infiltrating lymphocytes (TILs) have been reported to be associated with increased therapeutic efficacy of trastuzumab in the (neo)adjuvant setting for HER2-positive breast cancer (BC). Subcutaneous (SC) trastuzumab has been observed to act at different immunologic levels than IV trastuzumab. Therefore, by modifying the modality of administration of trastuzumab, it could be possible to interfere with different pathways of the immune system and exert a favorable immunomodulation in HER2-positive BC.

Trial design: In this non-comparative, phase II, neoadjuvant, randomized study, patients will be treated with FEC chemotherapy (fluourouracil 500 mg/m²; epirubicin 75 mg/m²; cyclophosphamide 500 mg/m²) q21 for 3 cycles. Then, they will be randomly assigned in a 1:1 ratio to receive: docetaxel (75 mg/m²) plus pertuzumab (840 mg loading dose, then 420 mg) plus IV trastuzumab (8 mg/kg loading dose, then 6 mg/kg) q21 for 4 cycles (Group A) or, docetaxel plus pertuzumab plus SC trastuzumab (fixed dose of 600 mg) q21 for 4 cycles (Group B). After surgery, study patients will receive trastuzumab q21 x 14 cycles using the same formulation (SC or IV) of the preoperative phase.

Eligibility criteria: Patients must have previously untreated, T2-4d primary HER2-positive BC with no metastatic disease. Other inclusion criteria are: age 18 or older; ECOG performance status 0-1; availability of tumor tissue from diagnostic biopsy; normal left ventricular ejection fraction; normal organ and marrow function.

Specific aims: The main objective of this trial is to evaluate variations of host immune response parameters to either trastuzumab SC or trastuzumab IV given in combination with pertuzumab and chemotherapy as neoadjuvant treatment of patients with HER2-positive BC. Tumor samples obtained at diagnosis and at definitive surgery will be centrally analyzed for TILs. Blood samples will be also collected during study treatment for tumor-specific lymphocyte cell activity (TLA) analysis. Feasibility, efficacy, safety and health-related quality of life will be also evaluated.

Statistical methods: The primary endpoint is post-surgery pathologic TIL rate on residual disease. The threshold for classifying subjects with high TILs, or not, is defined as equal to 15%, according to the median TIL rate observed in primary HER2-positive tumors. Because this is a phase II study with 2 non-comparative arms, Simon’s optimal 2-stage design will be used for each of the 2 study groups. For each arm we assume: \( p_r = 0.4 \), expected rate of subjects with high TILs on residual disease, \( p_o = 0.1 \), lowest limit of the subject rate \( (alpha=0.05; beta=0.20) \).

Present accrual and target accrual: A total of 60 patients (first stage: 16 patients) will be enrolled from multiple institutions. From November 29, 2016 to June 11, 2017, 34 patients have been recruited.

Contact information: Dr. Antonino Musolino, MD, MSc, PhD; Medical Oncology Unit, University Hospital of Parma; Tel: +390521702316; Fax: +390521995448; e-mail: amusolino@ao.pr.it. Clinical Trials.gov: NCT03144947.
Body: Background. Recent clinical trial results indicate that it is more appropriate than ever to conduct de-escalation clinical trials looking at less chemotherapy for patients with relatively early stage HER2+ breast cancer, particularly those with so-called triple positive breast cancer (ER+PR+HER2+, or TPBC). TBCRC 006 showed that if hormone therapy is added to dual-HER2 blockade (lapatinib and trastuzumab) pathological complete response rate (pCR) can increase to 21% with another 22% having low residual disease (<1cm) with only 12 weeks of neoadjuvant therapy. After CLEOPATRA showed unprecedented improvement in OS comparing docetaxel trastuzumab and pertuzumab versus docetaxel and trastuzumab (15.7 mo median OS difference), we designed NEOADAPT to test the hypothesis that a flexible duration of neoadjuvant treatment based on clinical and radiographic response with an aromatase inhibitor coupled with pertuzumab and trastuzumab would have a >40% pCR rate. The potential impact of this trial is to provide more treatment options for women with early stage TPBC in a current environment when more than 40% of such patients are currently getting chemotherapy.

Trial Design. This single arm prospective cohort study is IRB approved and currently enrolling (NCT02689921). Intervention will be neoadjuvant aromatase inhibitor +/- LHRH agonist or oophorectomy if premenopausal with trastuzumab and pertuzumab in standard q 3-week dosing schedules. Duration of treatment will be determined by clinical exam, q 3mo dynamic breast MRI but no longer than 1 year maximum before surgery. Study ends upon definitive surgery. Duration of treatment will be 3 months after last radiographic CR seen on MRI barring progression or patient/physician choice.

Eligibility Criteria. Patients with stage I-II biopsy confirmed invasive breast cancer that is ER/PR+ and HER2+ by latest ASCO guidelines. Multifocal breast cancer is allowed on the provision that all lesions are biopsied and confirmed to also be TPBC and felt by the pathologist to be the same tumor. Age ≥18 yrs, ECOG PS 0-2, LVEF ≥50% at baseline. Rest of inclusion and exclusion criteria are typical for most studies in this setting.

Specific Aims. The primary endpoint is to document pCR rate. Secondary endpoints are to conduct an exploratory analysis of whether Mammaprint can identify patients who are more likely to obtain pCR or not and to describe sensitivity and specificity of breast MRI in predicting pCR.

Statistical Design. To identify a hypothesized pCR rate of >40% with 80% power, 32 patients will be enrolled. The Fleming two stage design will be implemented with stopping rules with the first stage of interim analysis done when the first 15 evaluable patients have surgical results. Further details will be provided in poster.

Present accrual and target accrual. At time of abstract submission 5 of 32 patients have been enrolled and the study is currently available at 2 Cancer Treatment Centers of America sites, Midwestern and Southeastern Regional Medical Center. Plan is to open the study at all 5 CTCA sites before end of 2017.

Contact information for those specifically interested in this trial. Principal investigator Eugene Ahn MD eugene.ahn@ctca-hope.com.
2017 San Antonio Breast Cancer Symposium

Publication Number: OT1-03-05

Title: The DETECT V-Study – Comparison of dual HER2-targeted therapy with trastuzumab plus pertuzumab in combination with chemotherapeutic or endocrine therapy in addition with CDK4/6 inhibition in patients with HER2-positive and hormone-receptor positive metastatic breast cancer

Tatiana Romashova¹, Arkadius Polasik¹, Thomas WP Friedl¹, Brigitte Rack¹, Marie Tzschaschel¹, Peter A Fasching², Florin-Andrei Taran³, Andreas Hartkopf³, Andreas Schneeweiss³, Volkmar Mueller⁴, Aktas Bahriye⁵, Klaus Pantel⁶, Franziska Meier-Stiegen⁶, Pauline Wimberger⁸, Wolfgang Janni¹, Tanja Fehm⁸ and Jens Huober¹. ¹University of Ulm, Ulm, Germany; ²Gynecology and Obstetrics, University Hospital Erlangen, Erlangen, Germany; ³Gynecology and Obstetrics, University Hospital Tuebingen, Tuebingen, Germany; ⁴National Center for Tumor Diseases, Heidelberg, Germany; ⁵Gynecology and Obstetrics, University Hospital Hamburg-Eppendorf, Hamburg, Germany; ⁶Gynecology and Obstetrics, University Hospital Essen, Essen, Germany; ⁷University Hospital Hamburg-Eppendorf, Hamburg, Germany; ⁸Gynecology and Obstetrics, Heinrich-Heine University Hospital Duesseldorf, Duesseldorf, Germany and ⁹Gynecology and Obstetrics, University Hospital Dresden, Dresden, Germany.

Body: Background: Metastatic breast cancer (MBC) is usually an incurable disease and maintenance of quality of life (QoL) is one of the main aims of therapy. In patients with HER2-positive MBC taxane-based chemotherapy in combination with dual HER2-targeted therapy with trastuzumab and pertuzumab, has shown significantly increased progression free survival and overall survival. Adverse events are well-known side effects of any cytostatic treatment and can seriously impact the patients' QoL. The synergistic combination of dual HER2-targeted therapy with trastuzumab and pertuzumab plus endocrine therapy might offer a better treatment option for these patients. Preclinical data and first clinical trial results suggest an additional benefit when a CDK4/6 inhibitor is added to the combination of endocrine therapy and anti HER2 therapy. DETECT V is a randomized phase III study comparing the safety and efficacy of trastuzumab plus pertuzumab in combination with either endocrine therapy or chemotherapy. In both treatment arms the CDK4/6 inhibitor ribociclib will be added.

Trial design and eligibility criteria: Patients are 1:1 randomized to receive dual HER2-targeted therapy with trastuzumab and pertuzumab combined with endocrine therapy and ribociclib or to chemotherapy with trastuzumab and pertuzumab followed by maintenance therapy with trastuzumab, pertuzumab, endocrine therapy and ribociclib when chemotherapy has stopped. The sample size calculations are based on the assumption that the probability of having an adverse event as defined by the modified adverse event score for patients with HER2 positive metastatic breast cancer in the chemotherapy arm is 86.3%. Based on this assumption, a minimum of 121 patients per treatment arm is required to detect a 25% decreased risk of having an adverse event as defined by the modified adverse event score (i.e. a relative risk ratio of 0.75) for patients treated with dual HER2-targeted plus endocrine therapy and ribociclib as compared to patients treated with dual HER2-targeted plus chemotherapy with the combination of endocrine therapy and ribociclib as maintenance treatment (90% power, two-sided test, α = 0.05).

Specific aims: The primary objective of this study is to assess the tolerability of both treatment strategies, as assessed by the occurrence of AEs during the treatment period. Modified adverse event score was developed in order to better reflect the clinical, physiological and psychological impact of AEs on patients' QoL. Key secondary endpoint, besides the efficacy endpoints progression free survival (PFS) and overall survival, is to compare quality-adjusted survival (QAS), as measured using the quality-adjusted time without symptoms and toxicity (Q-TWiST) method, between both treatment arms. The DETECT V trial comes along with a comprehensive translational program focusing on detection and phenotyping of circulating tumor cell (CTC)-and the assessment of marker expression on CTCs in order to calculate an endocrine responsiveness score.
Title: Phase IB/II clinical trial to evaluate safety and efficacy of tucatinib in combination with palbociclib and letrozole in patients with hormone receptor positive and HER2-positive metastatic breast cancer

Elena Shagisultanova¹, Jennifer Diamond¹, Alison Stopeck², Lajos Pusztai³, Ruth O'Regan⁴, William Gradishar⁵, Ursa Brown-Glaberman⁶, Pavani Chalasani⁷, Tessa McSpadden¹, Michelle Borakove¹, Troy Shedin¹, Peter Kabos¹ and Virginia Borges¹. ¹University of Colorado Denver, Aurora, CO; ²Stony Brook University, Stony Brook, NY; ³Yale Cancer Center, New Haven, CT; ⁴University of Wisconsin, Madison, WI; ⁵Northwestern University, Chicago, IL; ⁶University of New Mexico Cancer Care Alliance, Albuquerque, NM and ⁷University of Arizona Cancer Center, Tucson, AZ.

Body: Breast cancers overexpressing HER2-oncogene and hormone receptors (HR) represent therapeutic challenge because of a bi-directional cross-talk between HR and HER2 pathways leading to tumor progression and drug resistance. There is a strong rationale for evaluation of novel targeted drug combinations in this breast cancer subtype.

We designed a phase IB /II clinical trial to test the combination of novel oral HER2 small molecule inhibitor tucatinib with CDK4/6 inhibitor palbociclib and aromatase inhibitor letrozole in patients with HR+/HER2+ metastatic breast cancer (NCT03054363). In addition to the rationale for the synergy of targeting HR, HER2 and CDK4/6 pathways simultaneously in this disease setting and its potential for anti-tumor efficacy, we propose this novel combination of three oral agents, if well tolerated, will be highly patient-centered as an effective non-chemotherapy based regimen for treatment of HR+/HER2+ breast cancer.

This multicenter clinical trial is conducted through the Academic Breast Cancer Consortium (ABRCC), with the University of Colorado Cancer Center as the lead site.

Target enrollment: 40 patients (20 patients in phase IB and 20 patients in phase II part).

Main inclusion criteria:
1. HR+/HER2+ locally advanced unresectable / metastatic breast cancer
2. Measurable or evaluable disease. Bone only disease is allowed.
3. Subjects without brain metastases are eligible; subjects with untreated asymptomatic CNS metastases not needing immediate local therapy, and subjects with stable brain metastases previously treated with radiation therapy or surgery are eligible
4. ECOG 0-1
5. Postmenopausal women, or premenopausal women on ovarian suppression
6. Prior treatments:
   - At least two approved HER2-targeted agents (trastuzumab, pertuzumab, or TDM-1) at any time in the course of the disease
   - At least 1 line of HER2-targeted therapy in the metastatic setting (with the exception of asymptomatic subjects with oligometastatic or bone / soft tissue only disease who, on investigator opinion, are appropriate for a front line single agent anti-endocrine therapy per NCCN guidelines)
   - Up to 2 lines of prior endocrine therapy in the metastatic setting are allowed
7. Adequate organ and marrow function

Main exclusion criteria:
1. Previously treated progressing brain metastases
2. Brain metastases and contraindications to undergo contrast brain MRI
3. Toxicities of prior cancer therapies that have not resolved to grade 1 or less, except peripheral neuropathy, which must have resolved to grade 2 or less, and alopecia
4. Previous treatment with EGFR or HER2 tyrosine kinase inhibitors or CDK4/6 inhibitors
5. Systemic anti-cancer therapy or radiation within 2 weeks of the first dose of study drugs
6. Active bacterial, fungal or viral infections, hepatitis B, C, or HIV
7. Clinically significant cardio-vascular disease

Primary objectives:
- Phase IB: safety and tolerability of combination therapy
- Phase II: efficacy by PFS

Exploratory assessment of biomarkers of resistance and response will be performed in the blood and biopsy samples

Study contact: Elena Shagisultanova, MD, PhD, elena.shagisultanova@ucdenver.edu
2017 San Antonio Breast Cancer Symposium

Publication Number: OT1-03-07

Title: Single arm, phase IIA clinical trial assessing the safety and activity of atezolizumab in combination with paclitaxel, trastuzumab, and pertuzumab in patients with metastatic HER-2 positive breast cancer (mHER2+BC)

Lori J Goldstein¹ and Elias Obeid¹. ¹Fox Chase Cancer Center, Philadelphia, PA.

Body: Background: HER2+BC is a subtype of breast cancer where tumor cells overexpress the HER2-receptor tyrosine kinase or have over-amplification of HER2/neu gene and comprises 25-30% of all BC. Pertuzumab and trastuzumab, are monoclonal antibodies that target HER2 signaling, and synergize to improve outcomes when added to a taxane regimen in mHER2+BC [CLEOPATRA trial]. Significant challenges persist as 15% of patients would relapse due to resistance to HER2-targeted therapy. Trastuzumab has an immune-mediated activity to sensitize HER2-overexpressing tumors to the killing by cytotoxic T lymphocytes (CTLs). A positive correlation between T-cell infiltration (TILs) and outcomes in HER2+BC has been reported. Atezolizumab is a humanized IgG1 monoclonal antibody targeting human programmed death ligand 1 (PD-L1) and inhibits its interaction with its receptor PD-1. Atezolizumab also blocks binding of PD-L1 to B7.1, an interaction conferring additional inhibitory signals to T cells. Certain chemotherapeutic drugs, such as taxanes, mediate their anticancer activity not only by direct cytotoxic effects, but also by activation of CD8+ T-cell responses. Here we propose a single arm, Phase IIA clinical trial to study the safety and efficacy of atezolizumab in combination with a standard regimen of paclitaxel, trastuzumab and pertuzumab in patients with mHER2+BC, in comparison with a historic cohort of the same regimen without atezolizumab.

Methods: A total of 50 subjects will be enrolled. Premedication systemic corticosteroids are usually administered with taxane therapy to avoid hypersensitivity reactions. With concerns over a negative immune effect from corticosteroids on atezolizumab activity, this protocol's subjects will receive premedication with dexamethasone only for weeks 1 and 2 of weekly paclitaxel, and then discontinued if there is no hypersensitivity reaction. The primary endpoint is safety and efficacy (overall response rate) by evaluation for acute toxicity and of tumor response using RECIST v1.1. Other endpoints include clinical benefit rate (CBR), progression-free survival (PFS), overall survival (OS), and duration of response (DOR). Tumor biopsies and peripheral blood collection at baseline and just prior cycle 4 are mandatory for correlative studies. PD-L1 expression will be evaluated in exploratory analysis with a planned assessment of response based on PD-L1 status as well as by hormone receptor status. NCT03125928 www.clinicaltrials.gov
Title: A multicenter, phase I/II trial of anastrozole, palbociclib, trastuzumab and pertuzumab in HR-positive, Her2-positive metastatic breast cancer

Krystal P Cascetta¹, Poulikos Poulikakos¹, Charles Shapiro³, Julie Fasano¹, Aarti Bhardwaj¹, Hanna Irie¹, Anupama Goel¹, Paula Klein¹, Sylvia Adams², Kevin Kalinsky³, Linda Vahdat⁴, Meng Ru¹ and Amy Tiersten¹. ¹Mount Sinai Hospital and Icahn School of Medicine, New York, NY; ²Perlmutter Cancer Center NYU Langone, New York, NY; ³New York Presbyterian Herbert Irving Comprehensive Cancer Center at Columbia University, New York, NY and ⁴New York Presbyterian Meyer Cancer Center at Weill Cornell, New York, NY.

Body: Background: Overexpression or amplification of HER2 occurs in approximately 15 – 20% of patients and about half of these tumors are hormone receptor (HR) positive. Studies suggest that this 10% of all breast cancer cases may derive less benefit from endocrine therapy than those with HR positive disease without HER2 overexpression. The use of aromatase inhibitors in the metastatic setting is well established while significant improvement in overall survival has been established with the use of trastuzumab or pertuzumab in HER2-overexpressing tumors. To date, no studies have examined the combination of endocrine therapy, palbociclib, and dual HER2 therapy with pertuzumab and trastuzumab in this patient population.

Trial Design: Multicenter, Phase I/II Trial of Anastrozole, Palbociclib, Trastuzumab and Pertuzumab in HR+, HER2+ metastatic breast cancer.

Eligibility Criteria: Stage IV hormone receptor positive, HER2 positive breast cancer patients.

Specific Aims: Phase I: To determine the maximum dose tolerated of palbociclib. Phase II: To determine the clinical benefit rate (CBR) of treatment with anastrozole, palbociclib, trastuzumab, and pertuzumab in HR+, HER2+ metastatic breast cancer patients. Exploratory: Examine potential biomarkers of response to palbociclib including cyclin D1 expression levels, phosphorylated retinoblastoma expression and p16 levels. RNA sequencing will be used to assess for other predictors of response in an unbiased manner to see if there is correlation with inhibition of Ki-67 and phosphorylated retinoblastoma expression as well as evaluate for potential mechanisms of resistance.

Statistical Methods: This study will evaluate the maximum tolerated dose (MTD) of the Anastrozole, Palbociclib, Trastuzumab and Pertuzumab. If ≥ 33% of patients experience a dose limiting toxicity (DLT) at any dose level, the dose level below that level will be considered the MTD. Or, if the highest level has been reached and < 33% of patients have experienced DLT, that will be considered the MTD. Once the MTD is reached, we will assess the clinical benefit rate using a Simon's II stage design among a maximum 30 patients.

Accrual: Maximum of 36 subjects.
Estetrol for treatment of advanced ER\(^+\) breast cancer

Carole Verhoeven\(^1\), Marcus Schmidt\(^2\), Arnd Höning\(^3\), Jan Dünnebacke\(^4\), Ellen Dutman\(^1\), Jan Krijgh\(^1\) and Herjan Coelingh Bennink\(^1\).

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Background information

Estrogen therapy was the endocrine treatment of choice in postmenopausal women with advanced breast cancer for several decades since 1944. In the 1970s, estrogen therapy was replaced by tamoxifen. Tamoxifen showed similar regression rates but less toxicity as compared to estrogen therapy and was therefore considered preferred. Recently, estrogen therapy has gained new interest as several clinical studies showed clinical benefit in heavily pre-treated patients with advanced breast cancer after long-term estrogen deprivation. Estrogen therapy is an effective treatment for breast cancer but it has a negative safety reputation, especially related to the cardiovascular (CV) system. The fetal estrogen estetrol (E4) might be a new treatment option for patients with advanced breast cancer. It has less interference with liver function and is expected to be less harmful for the CV system compared to other estrogens whereas data from non-clinical and clinical studies suggest anti-tumor effects of E4 in breast tumors.

Hypothesis

The fetal estrogen E4 may have anti-tumor effects in patients with advanced ER\(^+\) breast cancer. It will most likely also reduce symptoms and signs of estrogen deficiency such as hot flushes, arthralgia, sleep disturbances, bone loss and cognition and improve patient's quality of life.

Trial design and methods

This is a multi-center, open-label, phase I/IIa, dose-escalation study with a 3 + 3 cohort design to determine the recommended dose of E4 for the treatment of patients with advanced breast cancer. Cohorts of at least 3 patients will receive doses of 20 mg, 40 mg and 60 mg E4 respectively, until the non-tolerable dose is determined or the maximum dose of 60 mg is reached. Patients will be treated once daily by oral administration for 12 weeks. In total 9-18 patients with advanced ER\(^+\) breast cancer will be enrolled at three centers in Germany.

Objectives

The main objectives of this study are to evaluate the safety, the effects on estrogen deficiency symptoms and the preliminary anti-tumor activity of E4 in patients with advanced breast cancer.

Eligibility criteria

Women who are more than 5 years postmenopausal, with ER\(^+\) and HER2-negative locally advanced and/or metastatic breast cancer are eligible for inclusion. In addition, patients should have failed on anti-estrogen treatment with tamoxifen and aromatase inhibitors. Patients with a history of venous or arterial thromboembolic disease or a known defect in the blood coagulation system will be excluded as well as patients who have used treatment with fulvestrant within 6 months prior to start of E4 treatment.

Statistical methods

Statistical tests used in this study are exploratory only. No confirmatory statistical test procedure is envisaged.

Contact information

For further information regarding the study, please contact Dr Carole Verhoeven (cv@pantarheibio.com).
Title: Mifepristone treatment for breast cancer patients expressing levels of progesterone receptor isoform A (PRA) higher than those of isoform B (PRB)

Marcos Liguori¹, Claudia Lanari², Hugo Gass¹, Paola Rojas², Andres Elia², Paula Martinez Vazquez¹, Javier Burruchaga¹, Pedro Gonzalez¹, Ines Caillet Bois¹, Carla Ventura¹, Gustavo San Martin¹, Alejandra Castets¹, Silvia Lovisi¹, Gabriela Acosta Haab³, Caroline Lamb², Victoria Fabris², Virginia Novaro² and Alfredo Molinolo⁴. "Magdalena V. Martinez Hospital, Pacheco, Tigre, Buenos Aires, Argentina; Centro de Investigación y Desarrollo en Investigación y Producción, Ciudad Autonoma, Buenos Aires, Argentina; San Isidro Patologia, San Isidro, Buenos Aires, Argentina and Moores Cancer Center, San Diego, CA.

Body: Seventy percent of breast cancers express estrogen (ER) and progesterone receptors (PR) and respond to antiestrogen therapies. Emerging evidence from experimental studies and human epidemiology, points to a relevant role for progestins in breast carcinogenesis and cancer growth. Others and we have proposed that there is a role for antiprogestins in the therapeutic armamentarium, but the challenge remains to identify which patients would benefit from targeting the PR in addition to ER. Preclinical data indicates that antiprogestins block cell proliferation and increase apoptosis only in ER+ breast cancers expressing levels of PRA higher than those of PRB. The aim of this study is to evaluate the therapeutic effects of Mifepristone (MFP) on breast cancer patients selected by their PRA/PRB isoform ratio, for 14 days in between core biopsy and surgery (MIPRA trial ClinicalTrials.gov Identifier: NCT02651844). Methods: This is an open label, interventional with single group assignment study. We perform core biopsies on menopausal patients with clinically palpable breast cancers larger than 1.5 cm to confirm diagnosis. We will assess the PRA/PRB ratio by western blotting in frozen samples and total PR in formalin-fixed samples by immunohistochemistry (IHC). Twenty eligible PR+ patients (PR > 50 %) with PRA/PRB ≥1.5 who have signed consent forms, and meet the inclusion criteria will be recruited. Patients will be treated for 14 days with MFP p.o 200 mg. Surgery will be performed on day 15. Samples will be frozen for molecular studies or fixed for IHC. The primary outcome is the evaluation of Ki-67 staining pre- and post treatment. Secondary outcomes include comparatively expression of proliferation/apoptosis/PR signaling markers in core and surgical biopsy samples. Other pre-specified outcomes include molecular profiling, study of liquid biopsies, mammography, and ultrasound studies. Wilcoxon signed rank test will be used to evaluate differences in biomarker expression between core biopsy and surgical samples of each patient.
Title: A phase I/II, single arm, non-randomized study of ribociclib (LEE011), a CDK 4/6 inhibitor, in combination with bicalutamide, an androgen receptor (AR) inhibitor, in advanced AR+ triple-negative breast cancer

Cesar A Santa-Maria¹, Murtuza Rampurwala², Kari Wisinski³, Deborah Toppmeyer⁴ and Ruth O'Regan³. ¹Northwestern University; ²University of Chicago; ³University of Wisconsin and ⁴Rutgers University.

Body: Background: Triple negative breast cancer (TNBC) is a heterogeneous disease encompassing distinct intrinsic molecular subtypes, including a luminal androgen receptor (AR) subtype, characteristically dependent on AR signaling. The AR is expressed in more than 50% of TNBCs. Bicalutamide is an oral, non-steroidal, AR antagonist, which has been studied in metastatic TNBC with a clinical benefit rate of 19% at 24 weeks. In preclinical models, cyclin dependant kinase (CDK) 4/6 inhibition has been shown to restore sensitivity to AR inhibition, and may thus be an important resistance mechanism. Ribociclib is an orally bioavailable, highly specific CDK4/6 inhibitor that induces cell cycle arrest, already approved in endocrine receptor positive breast cancers. We hypothesize that inhibition of CDK inhibition can enhance the activity of anti-androgen therapy in TNBC that express AR.

Methods: We designed a phase I/II, single arm, non-randomized, open label study of the combination of bicalutamide with ribociclib in women with advanced AR-positive TNBC. The primary objective of the phase I component is to determine the maximum tolerated dose of the combination, and of the phase II component to assess the clinical benefit rate at 16 weeks. Secondary objectives include progression free and overall survival, objective response rates, and safety and tolerability. Exploratory objectives will be to assess AR quantification, localization and splice variants in circulating tumor cells, as well as quantification of pan and phospho proteins of Rb. Eligible patients must have measurable metastatic or unresectable AR-positive TNBC and have had no more than 1 line of systemic therapy for metastatic disease. The phase I study will be conducted using a 3+3 dose escalation schema, 12 to 18 patients are expected to enroll. The phase II component will utilize a Simon's two stage design, enrolling 24 patients for the first stage. At least 5 subjects must have clinical benefit by 16 weeks to proceed onto the second stage, which would enroll an additional 22 subjects for a total of 46 patients. The study will be powered to detect a clinical benefit rate of 40% with a power of 80% and a type I error rate of 10%. Contact dmusapatika@hoosiercancer.org for more information about the study.
Title: A phase II clinical trial of the combination of pembrolizumab and selective androgen receptor modulator GTx-024 in patients with advanced androgen receptor positive triple negative breast cancer

Yuan Yuan¹, Paul Frankel¹, Timothy Synold¹, Peter Lee¹, Susan Yost¹, Norma Martinez¹, Aileen Tang¹, Blanca Mendez¹, Daniel Schmolze¹, Sophia Apple¹, Arti Hurria¹, James Waisman¹, George Somlo¹, Niki Tank¹, Mina Sedrak¹ and Joanne Mortimer¹. ‘City of Hope Comprehensive Cancer Center and Beckman Research Institute, Duarte, CA.

Body: Background: Androgen receptor (AR) targeted therapy and immunotherapy represent one of the most promising strategies for metastatic triple negative breast cancer (mTNBC), which accounts for 15-20% of all breast cancers. As a nonsteroidal selective androgen receptor modulator (SARM), GTx-024 demonstrated preclinical activity in AR⁺ TNBC PDX model. Pembrolizumab is a highly selective humanized monoclonal antibody of the programmed cell death 1 receptor (PD-1). The complementary modes of action and low potential for overlapping toxicity make the combination promising in patients with AR⁺ mTNBC.

Trial Design: This is an open-label Phase 2 study for AR⁺ mTNBC. Eligible participants receive pembrolizumab 200mg IV every 3 weeks in combination with GTx-024 18mg po daily.

Eligibility Criteria: Eligible patients must have AR⁺ (>10%, 1+ by IHC) TNBC; failed up to 2 lines of therapy in metastatic setting; and have measurable disease per RECIST1.1. Patients are excluded if they have had prior checkpoint inhibitors or AR targeted agents. Patients with current or prior use of testosterone, testosterone-like agents, androgenic compounds, or anti-androgens (including systemic steroids and immunosuppressive medications) are excluded, as well as current or prior history of noninfectious pneumonitis requiring systemic steroid therapy.

Specific Aims: The primary objective is to evaluate the safety/tolerability of GTx-024 and pembrolizumab and determine the response rate (CR or PR via RECIST 1.1) in patients with advanced AR⁺ TNBC. We will use clinical benefit rate (CBR), duration of response (DOR), PFS, and OS to test the efficacy of this novel drug combination.

Statistical Design: A Simon's MiniMax two-stage Phase 2 design will be utilized. Based on the previously reported response rate associated with single agent pembrolizumab (19%), we consider a response rate of 19% for the combination as discouraging, and a 39% response rate as encouraging. As a result, we will initially accrue 15 patients (including 6 patients from safety lead-in treated at the tolerable dose). If 2 or fewer patients respond, we will stop accrual for futility. Otherwise, the study will accrue an additional 14 patients for a total of 29 patients. With 29 patients, if only 8 or fewer respond (≤27.6%), the study will be considered discouraging unless secondary evidence of clinical benefit is substantial. With more than 8 patients responding out of the 29 patients, the combination would be considered promising. This design has 85% power to declare a true response rate of 39% as promising (power), and a 10% probability of declaring a true 19% response rate as encouraging (type I error). The probability of early termination if the true response rate is 19% is 44%.

Target Accrual: 29

Study Contact: Yuan Yuan MD PhD, City of Hope Comprehensive Cancer Center; Duarte, CA 91030; Email: yuyuan@coh.org
TRIO030: A presurgical tissue-acquisition study to evaluate molecular alterations in human breast cancer tissue following short-term exposure to the androgen receptor antagonist darolutamide

John R Mackey, Wolfgang Eiermann, Rodrigo Fresco, Helena Fung, Stephanie Carrez, Celine Lopez and Dennis J Slamon.

University of Alberta, Edmonton, AB, Canada; Interdisciplinary Oncology Center, Munich, Bavaria, Germany; Translational Research in Oncology (TRIO), Montevideo, Uruguay; Statistics, Translational Research in Oncology (TRIO), Edmonton, AB, Canada; Project Management, Translational Research in Oncology (TRIO), Paris, Île-de-France, France; Clinical Start-Up Unit, Translational Research in Oncology (TRIO), Paris, Île-de-France, France and David Geffen School of Medicine at University of California Los Angeles, Los Angeles, CA.

Body: Background: Endocrine treatment remains a mainstay in the treatment of patients with estrogen-receptor (ER) positive breast cancer (BC). Although ER and progesterone receptor (PgR) are well established targets with predictive and prognostic value, the clinical significance and role of the androgen receptor (AR) is poorly understood. Darolutamide is a new AR antagonist developed for the treatment of castration-resistant prostate cancer. Darolutamide potently inhibits testosterone binding to AR and retains antagonistic properties in cells expressing increased AR levels. Understanding the biological impact of AR-blockade when darolutamide targets the AR in BC should provide foundational rationale for its development in BC.

Trial Design: TRIO030 is a multi-center, open-label, tissue-acquisition study involving 60 women with early-stage invasive BC. To be able to assess the molecular alterations after darolutamide exposure in different BC subtypes, BC patients with triple-negative, or ER+/HER2 negative, or HER2 positive disease will be enrolled in the study, with 20 subjects in each group. Each participant will receive darolutamide at a dose of 600 mg b.i.d. (daily dose of 1200 mg) for a minimum duration of 14 days until the day prior to BC surgery (recommended duration of 21 days, maximum 35 days). Tumor tissue collection will include 3 pre-treatment cores (2 snap-frozen and 1 paraffin embedded) and 2 cores at surgery (1 snap-frozen and 1 paraffin embedded).

Key Eligibility Criteria: Eligible female subjects will have 1) histologically proven invasive breast carcinoma for which surgery is indicated as the primary treatment modality, 2) known ER, PgR and HER2 statuses, 3) tumor confined to either the breast or to the breast and ipsilateral axilla with T1 ≥ 1.5cm, T2 or T3 and either clinically positive (N1 only) or clinically negative axillary nodes (N0), and 4) no prior systemic nor local treatment for BC.

Study Objectives: The primary objective of this study is to identify the molecular changes that occur in BC tissue following short-term exposure to darolutamide in women with early BC. Secondary objectives include the safety and tolerability of short-term darolutamide in women with early BC.

Statistical Methods: Evaluability of subjects will require minimum duration of 10 consecutive days of treatment with darolutamide, tumor tissue collection at screening and at surgery and adequacy of tumor tissue samples for molecular assessment as determined by the central laboratory. Molecular analyses will be conducted on the evaluable population. Descriptive statistics will be used to compare pre and post treatment molecular findings. Correlations will be sought among molecular alterations from tumor samples collected at baseline, following administration of darolutamide, and clinicopathologic variables.

Accrual: The study will be activated in June 2017. Up to 60 evaluable subjects will be enrolled within 12 months (NCT03004534).
2017 San Antonio Breast Cancer Symposium

Title: OPTIMA: A prospective randomized trial to validate the predictive utility and cost-effectiveness of gene expression test-directed chemotherapy decisions in early breast cancer

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Body: Background: Multi-parameter gene expression assays (MPAs) are widely used to estimate individual patient residual risk in hormone-sensitive HER2-negative node-negative early breast cancer, allowing patients with low risk to safely avoid chemotherapy. Evidence for MPA use in node-positive breast cancer is limited. OPTIMA (Optimal Personalised Treatment of early breast cancer using Multi-parameter Analysis) aims to validate MPA's as predictors of chemotherapy sensitivity in a largely node-positive breast cancer population.

Methods: OPTIMA is a partially blinded multi-center, phase 3 randomized controlled trial with an adaptive two-stage design. The main eligibility criteria are women or men aged 40 or older with resected ER-positive, HER2-negative breast cancer and up to 9 involved axillary lymph nodes. Randomization is to standard management (chemotherapy and endocrine therapy) or to MPA-directed treatment. Those with a “high risk” tumor MPA score receive standard management whilst those at “low risk” are treated with endocrine therapy alone. The preliminary phase (OPTIMA prelim) evaluated the performance of several MPAs to select a test to be used in the main efficacy trial based on economic analysis, and assessed the feasibility and acceptability of a large UK trial. OPTIMA prelim used Oncotype DX as the primary discriminator; the main trial will use Prosigna (PAM50) with Prosigna Score ≤60 defined as “low-risk”. The co-primary outcomes are (1) Invasive Disease Free Survival (IDFS) and (2) cost-effectiveness of test-directed therapy. Secondary outcomes include IDFS in “low-risk” patients, quality of life and additional survival measures. An integrated qualitative recruitment study will identify and address challenges to recruitment and informed consent. Tumor blocks from all consenting participants will be banked allowing the performance of alternative MPA technologies to be evaluated. Recruitment of 4500 patients will permit demonstration of 3% non-inferiority of test-directed treatment, with 5% significance and 85% power, assuming 3 years follow-up and a control arm 5-year IDFS of at least 85%. The addition of patients from OPTIMA prelim will allow non-inferiority to be assessed with 2.5% significance.

Results: OPTIMA-prelim recruited 412 patients in 23 months from 35 sites with a 47% acceptance rate. The main study opened in January 2017. Early progress indicates that the recruitment target is achievable in the intended 46-month timescale through the participation of >100 sites

Conclusion: OPTIMA, as one of two large scale prospective trials validating the use of test-guided chemotherapy decisions in node-positive early breast cancer, is expected to have a global impact on breast cancer treatment. Experience from OPTIMA prelim showed that patient advocate support and close engagement with sites will aid trial success.

Funding: The project is funded in the UK by the NIHR HTA Programme (10/34/501). Views expressed are those of the authors and not those of the HTA Programme, NIHR, NHS or the DoH.
Title: Oncotype DX®-REMAR(Rhein-Main-Registry)-study: Use of the oncotype DX® assay in early breast cancer in certified breast cancer centers in Rhine-Main Region, Germany

Louiza Anastasiadou1, Sebastian Aulmann2, Stephan Falk2, Peter Baier3, Dagmar Giesecke4, Stefanie Buchen5, Ursula Hurst6, Eckardt Krapfl7, Voelker Moebus8, Dietrich Mosch9, Elke Schulmeyer10, Christine Solbach11, Sven Ackermann12, Boris Gabriel13, and Christian Jackisch14. 1AGAPLESION, Markus Hospital, Frankfurt Main, Hessen, Germany; 2OptiPath, MVZ für Pathologie, Frankfurt Main, Hessen, Germany; 3Ketteler Hospital Offenbach; 4Hochtaunus-Hospital, Bad Homburg; 5Asklepios Paulinen Klinik, Wiesbaden; 6KKH Bergstrasse, Heppenheim; 7Asklepios Klinikum Langen; 8Klinikum Frankfurt Hoechst; 9Bad Soden Klinik; 10Main-Kinzig-Kliniken, Gelnhausen; 11University Hospital Frankfurt; 12Klinikum Darmstadt; 13St. Josefs Hospital, Wiesbaden and 14Sana Klinikum Offenbach.

Body: A brief background discussion:
The OncotypeDX® multigene assay is recommended by several international guidelines as a predictive and prognostic factor for HR+/HER2- early breast cancer (EBC). Several trials have shown the necessity of OncotypeDX® Recurrence Score (RS) as a decision tool for HR+/HER2- EBC with 0-3 lymph nodes (LN) involved. At present, though, only limited data from the routine setting are available on the impact of treatment decision making process based on the usage of classical proliferation marker Ki67 with or without knowing the individual RS prior and after a treatment decision from a multidisciplinary tumor board (MTB) for the decision making of the adjuvant therapy of EBC. The Oncotype DX® assay is still not reimbursed by every insurance, therefore we expect that this registry will have an impact on reimbursement in Germany.

Trial design:
The OncotypeDX®-REMAR(Rhein-Main-Registry) study is a prospective, non-interventional, multicenter and non-randomized, study. 13 certified breast cancer (BC) centers in the Rhine-Main region in Germany participate. The sponsor of this trial is the AGAPLESION, Markus Hospital Frankfurt, Genomic Health provides the financial support.

After registration, the patient’s case will be discussed in the respective institution's MTB, before and after the RS result. The adjuvant treatment will be recommended based on available clinical and histopathological data according to the guidelines and the RS result. After each meeting, the physician and patient will fill in a questionnaire. In addition to the local determination of Ki67 and nuclear grading, a central pathology assessment of these two markers will be provided in a blinded fashion. Only the sponsor has access to these results. Consequently, this independent test has no influence both on the local histopathology result and on the recommended treatment.

Eligibility criteria:
Inclusion criteria:
Female patients, ≥18 years, with a hormone-receptor positive, HER2-negative EBC and 0-3 positive LN, T1-3, nuclear grading 1-3, Ki67:10-40%, cM0.

Specific aims:
Primary endpoint is the decision impact of the RS result in patients with mid-range Ki67 on adjuvant chemotherapy in EBC. Secondary endpoints include the correlation of Ki67 with tumor grade and RS result. Moreover, an economic subanalysis will be done.

Statistical methods:
The change in physicians’ treatment recommendations will be measured pre-assay vs. post-assay. The proportion of patients for whom the treatment recommendation changed and the 95% confidence interval will be reported overall and by select groups. McNemar’s test will be used to compare the proportion of patients’ recommended chemo-hormonal therapy pre-assay vs. post-assay.

Present accrual and target accrual:
Thirteen participating centers recruited 97 patients by the end of April 2017. 600 patients are planned in total.

Contact information for people with a specific interest in the trial:
In case of interest you can contact Ms. Louiza Anastasiadou, Tel: +4969-9533-66395, Fax: +4969-9533-2385, email: louiza.anastasiadou@fdk.info
2017 San Antonio Breast Cancer Symposium

Publication Number: OT1-06-03

Title: The plasmaMATCH trial: A multiple parallel cohort, open-label, multi-centre phase II clinical trial of ctDNA screening to direct targeted therapies in patients with advanced breast cancer (CRUK/15/010)

Nicholas Turner¹,², Hannah Bye¹, Sarah Kernaghan³, Paula Proszek¹, Charlotte Fribbens¹, Laura Moretti³, James Morden³, Claire Snowdon³, Iain Macpherson⁴, Andrew Wardley⁵, Rebecca Roylance⁶, Richard Baird⁷, Judith Bliss³ and Alistair Ring⁸. ¹The Institute of Cancer Research, London, United Kingdom; ²The Royal Marsden NHS Foundation Trust, London, United Kingdom; ³The Institute of Cancer Research Clinical Trials & Statistics Unit (ICR-CTSU), London, United Kingdom; ⁴The Beatson West of Scotland Cancer Centre, Glasgow, United Kingdom; ⁵The Christie NHS Foundation Trust, Manchester, United Kingdom; ⁶NIHR University College London Hospitals Biomedical Research Centre, University College London Hospitals NHS Foundation Trust, London, United Kingdom; ⁷Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom and ⁸The Royal Marsden NHS Foundation Trust, Sutton, United Kingdom.

Body: Introduction
Circulating tumour DNA (ctDNA) is found in the plasma of over 90% of patients with advanced breast cancer (BC). Screening for the presence of mutations in ctDNA provides a current assessment of the genetic profile of the patient's recurrent BC. The plasmaMATCH trial is designed to assess the potential of ctDNA screening to direct targeted therapies in patients with advanced breast cancer.

Methods
plasmaMATCH is a multi-centre phase IIa umbrella trial platform of ctDNA screening and a therapeutic trial. The study will screen 1000 women with advanced breast cancer, who have received prior systemic treatment in the advanced setting, with digital PCR ctDNA assays for hotspot mutations in ESR1, HER2, AKT1, and PIK3CA, with HER2 copy number assessment, in a central laboratory. The study will recruit from up to 50 sites in the UK. Patients with mutations identified will enter the matching treatment cohort, ESR1 – extended dose fulvestrant 500mg every two weeks, HER2 – neratinib +/- fulvestrant, AKT1 – AZD5363 +/- fulvestrant.

Mutation prevalence is presented with corresponding exact 95% confidence intervals (CIs) both overall and excluding 14 patients who were known to have mutations from a prior screening program. Patients with more than one mutation are included once in each relevant row.

Results
We report the results of prospective ctDNA mutation testing in the first 92 patients. plasmaMATCH opened to recruitment on 15/12/2016. As of 08/06/2017, 120 patients have been registered for ctDNA screening from 7 UK centres, of which 92 have ctDNA screening results available:

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Prevalence (95% CI)</th>
<th>Prevalence excluding 14 patients with known mutations (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR1</td>
<td>34/92: 37% (27%-48%)</td>
<td>26/78: 33% (23%-45%)</td>
</tr>
<tr>
<td>HER2</td>
<td>5/90: 6% (2%-12%)</td>
<td>2/76: 3% (0%-9%)</td>
</tr>
<tr>
<td>AKT1</td>
<td>7/92: 8% (3%-15%)</td>
<td>4/78: 5% (1%-13%)</td>
</tr>
<tr>
<td>PIK3CA*</td>
<td>22/92: 24% (16%-34%)</td>
<td>21/78: 27% (18%-38%)</td>
</tr>
</tbody>
</table>

*No corresponding plasmaMATCH treatment cohort

14 patients had more than one mutation detected (10 ESR1+PIK3CA, 3 ESR1+AKT1, 1 ESR1+HER2+AKT1). ctDNA results were reported in a median of 8 working days.

Of the 40 patients with one or more actionable mutation, 15 have entered a cohort, 16 are being screened for entry into a cohort, 5 are currently receiving further systemic treatment prior to cohort entry and 4 will not enter a cohort. One additional patient has
entered a treatment cohort on the basis of a mutation detected in an alternative tumour sequencing initiative.

Conclusions
plasmaMATCH ctDNA demonstrates the feasibility and accuracy of ctDNA testing as a screening tool for patients with advanced BC, with a high rate of subsequent recruitment into matching therapeutic trials.
**Title:** [{\textsuperscript{18}}F] fluoroestradiol (FES) PET as a predictive measure for endocrine therapy in women with newly diagnosed metastatic breast cancer

Hannah Linden\textsuperscript{1}, Amy Clark\textsuperscript{2}, Amy Fowler\textsuperscript{3}, Alena Novakova\textsuperscript{1}, David Mankoff\textsuperscript{2} and Farrokh Dehdashti\textsuperscript{4}. \textsuperscript{1}University of Washington, Seattle, WA; \textsuperscript{2}University of Pennsylvania, Philadelphia, PA; \textsuperscript{3}University of Wisconsin, Madison, WI and \textsuperscript{4}Washington University, Saint Louis, MO.

**Body:** Background: The majority of metastatic breast cancer patients have estrogen-receptor positive (ER+) disease. Therapy options include cytotoxic chemotherapy and ER-directed therapy, including endocrine therapy with or without molecularly targeted agents such as CK4/6 inhibitors. ER-targeted therapy is most commonly given first-line due to improved tolerability. However, not all patients will respond to first-line endocrine therapy due to intrinsic endocrine-therapy resistance mechanisms as well as tumor heterogeneity. There are no current methods in standard practice to inform on either of these issues. F-18 fluorestradiol (FES), an estrogen analogue has been considered the most promising ER imaging agent and is widely studied in breast cancer. FES positron emission tomography (PET) evaluates multiple tumor sites simultaneously and, thus, can inform on tumor heterogeneity of ER expression, and can measure ER binding in primary and metastatic sites (e.g., lymph nodes, lung, bone, and soft tissue). Like tissue ER expression, FES positivity, as measured by standardized uptake value (SUV), has been shown to predict response to endocrine therapy with selective ER modulators or aromatase inhibitors in first-line therapy or salvage settings in small studies. Typically, a significantly higher tumor SUV was noted in responders compared with non-responders. Since FES uptake can provide a better assessment of ER expression across all sites of metastatic disease, FES may provide more expansive information on ER expression. Furthermore, preliminary studies examining FES-PET in metastatic breast cancer have suggested that baseline FES uptake may predict response to endocrine therapy.

Trial Design: This is a prospective trial for patients about to start first line endocrine therapy for advanced ER+ breast cancer. Participants will undergo an FDG-PET within six weeks of FES-PET. FES-PET and serum hormone level to be completed prior to treatment initiation. Patients may opt to have a 2\textsuperscript{nd} FES-PET for test-re-test of FES-PET.

Specific Aims: Primary Aim: To assess the relationship between ER expression measured by FES-PET/CT and clinical benefit (response plus stable disease) of newly diagnosed ER+ metastatic breast cancer to endocrine therapy. Secondary Aim: To assess the correlation between FES uptake in ER+ metastatic breast cancer and tissue ER expression. Eligibility: Patients must have confirmed ER+ HER2- metastatic breast cancer and planning to receive endocrine therapy with or without CK 4/6 inhibitors. Patient must NOT have a history of \textsuperscript{\textgreater}1 line of administered chemotherapy for metastatic disease and may not have received endocrine therapy for advanced disease. Prior endocrine or chemotherapy in the adjuvant setting is allowed.

Methods: Participants will undergo a F-18 fluorodeoxyglucose (FDG)-PET/CT within six weeks of FES-PET/CT. FES-PET/CT will be completed prior to treatment initiation. Patients may opt to have a 2\textsuperscript{nd} FES-PET for test-re-test of FES-PET/CT

Present and Target Accrual: A total of 13 out of 99 patients have been enrolled onto this imaging study.
Body: Background
The crucial point in making treatment decisions for breast cancer patients is the assessment of tumour aggressiveness. The established prognostic markers may be insufficient to stratify cancer patients into treatment relevant risk groups. Emerging evidence indicates that mechanical properties of cancer cells and their microenvironment that occur on a nanometre scale play a critical role in cancer invasion and metastases. Therefore, detecting these nanomechanical changes could serve as biomarker of cancer aggressiveness.

Trial design
We conduct a prospective, blinded study in a routine clinical setting. Using minimal invasive breast biopsies we measure the nanomechanical (stiffness) properties of human breast tissue with our atomic force microscope (AFM) based method known as ARTIDIS (Automated and Reliable Tissue Diagnostics). These properties can only be measured using fresh (non-fixed) tissue under physiological conditions (Custodiol transplant buffer). This novel method is based on the use of a micro-fabricated 20nm-sharp tip that indents several thousand individual locations across tissue specimens within 60-180 minutes. Each indentation effectively measures the stiffness of local structures (e.g. cancer cells, extracellular matrix) located under the tip. Thus we obtain a quantitative, biopsy-wide, nanomechanical profile. Post-AFM the same biopsy is used for routine histopathological diagnosis, the current diagnostic gold standard to which the nanomechanical profile is then correlated.

Eligibility criteria
All women undergoing a minimal invasive breast biopsy (core needle or vacuum assisted biopsy) at the breast centre of the University of Basel.
Exclusion criteria: age younger than 18 years, necrotic/disintegrated biopsy, and technical limitations

Specific aims
Our primary endpoint is to differentiate benign from cancerous breast lesions based on their nanomechanical properties. Our secondary endpoint is to subclassify biopsies with cancerous lesions into the current four main breast cancer subgroups (Luminal A, Luminal B, HER2+ and basal-like).

Statistical analysis
The full dataset will include all patients with valid AFM measurements. Primary analysis: the proportion of true positive results divided by the total number of patients with malignant tumour (sensitivity) will be estimated and presented together with its 95% confidence interval. The histological diagnosis of the same biopsy as analysed by AFM will serve as gold standard.

Present accrual and target accrual
Present accrual as of June 12, 2017: 200 breast tissue biopsies.
Target accrual is 508 biopsies. This will allow for a power of 0.8 and a sensitivity of 90%.

Contact information: Rosemarie.Burian@usb.ch
Title: Observational study of axilla treatment for breast cancer patients with 1 to 3 positive micrometastases or macrometastases in sentinel lymph nodes

Shigeru Imoto¹, Mari Saito Oba², Norikazu Masuda³, Takeshi Nagashima⁴, Noriaki Wada⁵, Tsutomu Takashima⁶, Masahiro Kitada⁷, Masaya Kawada⁸, Tetsu Hayashida⁹, Tetsuya Taguchi¹⁰, Tomohiko Aihara¹¹, Daishu Miura¹², Uhi Toh¹³, Masayuki Yoshida¹⁴, Sadatoshi Sugae¹⁵, Kiyiayasu Yoneyama¹⁶, Hiroshi Matsumoto¹⁷, Hiromitsu Jinno¹⁸ and Junichi Sakamoto¹⁹. ¹Kyorin University School of Medicine, Mitaka, Japan; ²Toho University; ³National Hospital Organization Osaka National Hospital; ⁴Chiba University Graduate School of Medicine; ⁵Tokyo Dental College Ichikawa General Hospital; ⁶Osaka City University Graduate School of Medicine; ⁷Asahikawa Medical University; ⁸KKR Sapporo Medical Center; ⁹Keio University School of Medicine; ¹⁰Kyoto Prefectural University of Medicine; ¹¹Breast Center, Aihara Hospital; ¹²Toranomon Hospital; ¹³Kurume University School of Medicine; ¹⁴Seirei Hamamatsu General Hospital; ¹⁵Yokohama City University Graduate School of Medicine; ¹⁶Kyoto Prefectural University of Medicine; ¹⁷Kurosawa Hospital; ¹⁸Teikyo University School of Medicine and ¹⁹Tokai Central Hospital.

Body: [Background] Axilla surgery in node-positive breast cancer is dramatically changing from axillary lymph node dissection (ALND) to sentinel node biopsy (SNB). From the results of ACOSOG Z0011, IBCSG23-01 and AMAROS trials, adjuvant therapy and regional node irradiation could reduce regional lymph node recurrence for sentinel node-positive breast cancer patients. However, optimal indication of SNB alone remains uncertain. Trial design: To evaluate the outcome of sentinel node-positive breast cancer patients, the Japanese Society for Sentinel Node Navigation Surgery (SNNS) conducted a prospective cohort study in 2013 (UMIN000011782, Jpn J Clin Oncol, p.876-9, 2014). [Eligibility criteria] For eligible patients, SNB was performed or scheduled after 1 January 2012. Then 1 to 3 positive micrometastases or macrometastases in sentinel lymph nodes are confirmed by histological or molecular diagnosis. Primary chemotherapy before or after SNB is also acceptable for registration. [Specific aims] The primary endpoint is the 5-year recurrence rate of regional lymph node in patients treated with SNB alone. The secondary endpoint is the 5-year overall survival rate of this cohort. Patients treated with SNB followed by ALND are also registered simultaneously to compare the prognosis. The propensity score matching (PSM) is used to make the distributions of baseline risk factors comparable. [Statistical method] Based on an estimated recurrence rate of 5% at 5 years among patients treated with SNB alone, 240 patients are needed to give a 80% power to reject the null hypothesis that the recurrence rate is 10% with a one-sided type I error rate of 2.5%. If we consider that some patients will be lost to follow-up or become ineligible, a total of 250 patients will be needed to comprise the sample. [Present accrual] Eight hundred and eighty patients who underwent SNB alone or SNB followed by ALND were registered from 27 participating institutes between 2013 and 2016. Data cleaning is being performed. Patient's background and PSM will be reported.
Title: A pilot study to evaluate preoperative localization of breast and axillary lesions in neoadjuvant patients 31-365 days prior to surgery

Mary K Hayes¹, Erica V Bloomquist¹ and Heather R Wright¹. ¹Memorial Healthcare System, Hollywood, FL.

Body: SCOUT standard of care preoperative localization has been used in over 300 breast cancer patients at our hospital and over 12,000 breast cancer patients in 100 US sites between June 2015-May 2017. The radiologist performs SCOUT localization with Mammography, Ultrasound or CT guidance 0-30 days prior to surgery. In this study, we will evaluate the performance of SCOUT over longer duration (31-365 days) to address the needs of patients who require neoadjuvant treatment prior to definitive surgery. Placement of SCOUT localization prior to treatment response, when the lesion is clearly visualized on imaging, should be more accurate. Successful neoadjuvant treatment, which results in a complete or partial response often renders preoperative image-guided localization by the radiologist a more difficult and less reliable procedure and can result in unintended larger, more disfiguring breast cancer surgery. This pilot study will assess whether placement of the SCOUT prior to neoadjuvant treatment allows for longer-term preoperative localization of breast/axillary lesions. Successful performance with no significant adverse events may prove valuable to future patients who will require fewer and/or less extensive preoperative and surgical procedures. Some value may also be provided to subjects as the targeting before tumor shrinkage is expected to be more accurate.

Trial design Radiologists perform SCOUT localization using Mammography, Ultrasound or CT guidance 31-365 days preoperatively. Surgeons use the SCOUT system to locate and excise the SCOUT and target tissue.

Eligibility criteria The study population consists of 25-35 adult surgical patient volunteers who plan to have definitive breast cancer surgery at our hospital after neoadjuvant treatment.

Subject Inclusion Criteria
· Female patient 18-90 years old
· Patient is willing and able to comply with all study procedures and follow-up (1-13 months)
· SCOUT localization is performed before neoadjuvant treatment begins.

Subject Exclusion Criteria
· Patient is pregnant, has a pacemaker or implantable defibrillators
· Patient has known or suspected nickel allergy
· Patient is receiving an investigational drug that could potentially confound assessment of adverse device events

Statistical methods
This pilot study will provide an estimate for the efficacy of the method. Simple statistics will be used to evaluate primary and secondary endpoint data.

Primary Endpoint
The primary endpoint of this study is successful surgery, defined as a SCOUT localization at the target lesion, which remains in place 31-365 days preoperatively, and can successfully be removed with target tissue during surgery.

Secondary Endpoints
· Number of days prior to surgery (31-365) that the SCOUT is placed by the radiologist to localize the target breast/axillary tissue.
· Success rate of preoperative SCOUT placement by radiologist and SCOUT stability (within 1 cm of the center of the target tissue) for 31-365 days.

ClinicalTrials.gov identifier: NCT03015649

Contact information Mary Hayes, MD
Title: A prospective randomized study comparing surgery using electrosurgical bipolar sealing devices and surgery using conventional electro-cautery

Kwang Hyun Yoon¹, Hyung Seok Park¹, Joo Heung Kim¹, Jee Ye Kim¹, Sung Mook Lim¹, Seung Il Kim¹ and Young Up Cho¹.
¹Yonsei University College of Medicine, Seoul, Republic of Korea.

Body: Background: Seroma and lymphorrhea are the most common complication following mastectomy and/or axillary lymph node dissection (ALND). The manifestation of seroma accelerates complications such as post-operative bleeding, wound infection, prolonged recovery period. Sealing blood vessels and lymph drainages adequately during mastectomy and/or ALND may play a main role in reducing complication. Therefore, adequate sealing vessels and lymphatics during the surgery is important to reduce seroma and lymphorrhea related complications in patients with breast cancer. Objective: This study aims to show that electrosurgical bipolar sealing device for mastectomy could provide clinical benefit in reducing seroma formation.

Design: ELBCE (ELectrosurgical Bipolar Devices VS Convention Electronicautery, NCT03166384) is a prospective, randomized, two-arm assignment controlled trial of application of electrosurgical bipolar sealing device for mastectomy in patients with breast cancer. Patients is randomized in 1:1 ratio to conventional suture and tie group or electrosurgical bipolar sealing devices group. Randomization is performed using opening sealed envelope before enrollment. The primary end point is to compare the total drainage volume until drain tube removal. The time to drain removal is also measured. The secondary end points is the total amount of aspiration from seroma after drain tube removal, and the frequency of seroma aspiration.

Statistical consideration: A sample size 44 subjects per each arm was planned to provide a 90% power to detect a 25% decrease of the total amount of drainage fluid after surgery by using an electrosurgical bipolar sealing device when compared to a conventional suture and tie technique. In our experience, the average total volume of fluid after conventional suture and tie technique is 220 mL, with a standard deviation of 80.

Eligibility criteria: Inclusion criteria are patients older than 20 years and those who planned to undergo total mastectomy and/or ALND. Exclusion criteria are bilateral breast cancer patients, male patients, patients who underwent ipsilateral axillary surgery or radiation therapy, recurrent breast cancer patients, patients without drain catheter, and patients with ductal carcinoma in situ who have not undergone ipsilateral sentinel lymph node biopsy or ALND.

Present accrual and target accrual: ELSEBA study has an accrual target of 88 patients. To date, 8 patients have been randomized.

Contact information: Dr. Hyung Seek Park, E-mail: hyungseokpark.md@gmail.com; imgenius@yuhs.ac, Telephone: +82-2-2228-2100
Body: Background/Objective: ACOSOG Z1072 demonstrated complete target tumor ablation in 92% of stage I invasive breast cancers treated with cryoablation prior to resection. We hypothesize that cryoablation can achieve complete tumor ablation and adequate local control in a selected population of women with early stage invasive breast cancer managed with cryoablation alone, without planned surgical resection.

Methods: The trial is a phase II, single-arm, multicenter study open to women age 50 and older with core needle biopsy-proven clinical stage I, T1, clinically node negative (N0), unifocal, hormone receptor positive, HER2/neu-negative invasive ductal carcinoma measuring ≤1.5 cm by mammography (MG), ultrasound (US), and MRI. Once registered, subjects will undergo US-guided cryoablation followed 6 months later by an US-guided core biopsy of the cryoablated lesion to confirm the absence of residual viable disease. Following cryoablation, subjects will also begin a minimum 5-year course of adjuvant endocrine therapy and serial MG, US, and MRI. Whole breast radiotherapy (WBRT) is optional for subjects age 70+ (stratum 1) and mandatory for women age 50-69 (stratum 2). Sentinel node biopsy (SNBx) is optional in both cohorts. All subjects found to have residual or recurrent disease will undergo standard surgical resection.

Study Schema

<table>
<thead>
<tr>
<th>Registration</th>
<th>Cryoablation</th>
<th>6 Months</th>
<th>Semiannual</th>
<th>Annual</th>
<th>Resection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age 50+, tumor size ≤ 1.5 cm, unifocal, N0, biopsy proven invasive breast cancer</td>
<td>Followed by endocrine therapy (all patients); WBRT (Stratum 2); SNBx (optional)</td>
<td>MG, US, MRI, needle biopsy; resection of residual cancer or serial follow up if absent</td>
<td>Unilateral US or MG X 3 years</td>
<td>Bilateral MG and MRI</td>
<td>Recurrent/residual breast or axillary cancer</td>
</tr>
</tbody>
</table>

The trial was activated in June 2016. The study is currently open at 6 sites with up to 30 sites planned. A total of 110 eligible subjects will be enrolled in each stratum for total accrual goal of 220. Enrollment is planned over 3 years followed by 5 years of follow-up.

Results: The primary endpoint of the study will be to determine the rate of complete tumor ablation in patients treated with cryoablation, defined as absence of residual viable carcinoma detected by core needle biopsy of the ablation site performed 6-months post-cryoablation. The secondary endpoint will be to determine the 5-year ipsilateral breast tumor recurrence rate in patients treated with cryoablation without subsequent resection. Local recurrence will be defined as recurrence of cancer within the index quadrant confirmed histologically by needle biopsy. Breast cosmesis (assessed by BCCT.core) and adverse events will also be evaluated.

Conclusions: If the FROST Trial is successful at achieving complete ablation in ≥ 80% of study subjects, the trial will provide an important foundation for establishing cryoablation as an alternative to conventional therapy in selected women with early stage invasive breast cancer.

If interested, please contact Pam Ellis, clinical coordinator, at (925)460-6080 or pellis@sanarus.com.
Title: Sentinel lymph node biopsy (SLNB) and targeted axillary surgery (TAS) by indocyaningreen (ICG) and a novel near-infrared color camera system - a prospectively randomised, multicenter study to avoid radioactivity in a time-/cost-saving procedure in primary breast cancer

Peter Kern¹, Rainer Kimmig², Mahdi Rezai³, Oliver Hoffmann², Ines Bücker² and Michael Braun⁴. ¹University of Bochum, Teaching Hospital St.Elisabeth’s Hospital, Bochum, Northrhine-Westfalia, Germany; ²University of Duisburg-Essen, University Hospital of Essen, Essen, Northrhine-Westfalia, Germany; ³Breast Center Düsseldorf Luisenkrankenhaus, Düsseldorf, Northrhine-Westfalia, Germany and ⁴Rotkreuzklinikum München, München, Bavaria, Germany.

Body: Background: Sentinel lymph node biopsy (SLNB) by radioactively labelled 99mTechnetium +/- patent blue is so far the current standard in SLN detection. However, it remains a time- and cost-consuming procedure requiring the availability of a nuclear medicine department and a precise coordination with the subsequent surgery. It could be desirable to empower surgeons to be independent from availability of a nuclear source and to spare patients from radioactivity. Indocyanine green (ICG) as a fluorescent coloring agent is already known as safe in diagnostics for heart, circulation, liver and eye disease and may represent a valid alternative to 99mTc and patent blue (PBD), especially as it does not cause aesthetic impairment of the breast, with ICG being only visible with near infra-red light.

Methods: This prospective, randomized study is a non-inferiority trial to evaluate ICG-fluorescence as an alternative to either 99mTc and/or patent blue dye for sentinel lymph node detection of primary breast cancer with and without neoadjuvant chemotherapy.

Patients, aged 18 - 80 years, with unilateral or bilateral, unifocal or multifocal/ multicentric primary breast cancer without signs of metastases and written consent are eligible for this study. ECOG status of 0-2 and life expectancy > 1 year is required. All BMI classes are admitted to the study, with predefined subgroups of a) <= 20 b) >20-30 c) >30-40 d) >40. Tumor stages included are a) Tis (>= 4 cm) b) T1 c) T2 and d) T3. ICG-guided SLNB may be applied in patients in the following settings: a) before neoadjuvant, b) after neoadjuvant chemotherapy.

Primary Endpoints: Rate of SLN detection by either of the methods in the following 3 arms of the trial:
Arm A: 99mTc + patent blue dye (PBD)
Arm B: 99mTc + indocyanine green (ICG)
Arm C: Indocyanine green (ICG)

Secondary Endpoints:
- Time to identify (TTI) sentinel lymph node(s) (min)
- Number of sentinel lymph nodes (SLN) and non-sentinel lymph nodes (non-SLN)
- Rate of concordance of detection by 99mTc+patent blue dye (PBD) vs. 99mTc + ICG
- Dose of radioactivity omitted in Arm C vs. Arm A & B

Results: This trial is in progress.
2017 San Antonio Breast Cancer Symposium

Publication Number: OT2-02-01

Title: Brazilian randomized study - Impact of preoperative magnetic resonance in the evaluation for breast cancer conservative surgery (BREAST-MRI trial)

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Body: Background: A precise preoperative evaluation of the tumor is essential to improve breast cancer surgical management. Currently, mammography associated with ultrasound and clinical exam are the standard techniques for evaluating extension and tumor localization. However, approximately one third of the lesions in patients eligible for conservative surgical treatment are misevaluated by these methods. Breast magnetic resonance imaging (MRI) has a high sensitivity (95-100%) in detecting invasive neoplasms, and is able to detect occult tumors, multifocal and/or multicentric disease, and contralateral breast cancer more accurately than mammography and ultrasound. Until now, there are only three randomized trials assessing the role of preoperative MRI. These trials have different designs and contradictory results. Trial design: BREAST-MRI is a randomized, open label, unblinded trial designed to compare the accuracy of breast MRI in the preoperative planning of surgical treatment of breast cancer to standard protocol (clinical exam of the breast, mammography and/or breast ultrasound) and the impact of breast MRI on breast cancer outcome. Patients are randomized on a 1:1 basis, stratified for mammary density, into two groups: 1)MRI group: patients are submitted to MRI and standard protocol 2)Control group: standard protocol. First phase: patient recruitment and data collection up until surgery. Second phase: follow-up for five years or until death. Eligibility criteria: women aged 18 years or older with breast cancer stages I to III candidates for conservative surgery (CC). Specific aims: The aim of this study is to evaluate the ability of MRI in selecting patients for conservative treatment of breast cancer. Primary outcomes are: false positive rates, false negative rates, positive predictive value and negative predictive value of MRI in breast cancer CC. Secondary outcomes are: rates of positive margins on pathological examination; reoperation rates; number of conversions to mastectomy; accuracy of MRI according to mammographic density, immunohistochemical subtype and histopathology of the tumor; rates of multicentricity, multifocality and bilateralality of tumors; disease-free survival after 3 and 5 years; and cost-effectiveness of breast MRI. Statistical methods: The calculated total case number for this trial is 372, assuming a recurrence rate of 10% for CC and 1% for mastectomies. The Shapiro-Whilk test will be used to verify if distribution of the quantitative variables follows normal distribution. The baseline population will be analyzed using the t-Student test, or the Mann-Whitney test when appropriate. To test the existence of a possible association between outcomes and the categorized characteristics, chi-square and Fisher's exact test will be performed. Disease progression will be reassessed at 3 and 5 years follow-up, in order to produce a log-rank Kaplan-Meier curve of survival. Present accrual and target accrual: In June 2017, randomizations are at approximately 90% of the target sample size.
Title: Multi-institution phase II trial of intraoperative electron beam radiotherapy boost at the time of breast conserving surgery with oncoplastic reconstruction in women with early-Stage breast cancer

Jose G Bazan1, Julie Stephens1, Alicia Terando1, Roman Skoracki1, Sohyun McElroy1, Jennifer Sexton1, Nilendu Gupta1 and Julia White1. 1The Ohio State University, Columbus, OH.

Body: Background: In women amenable to breast conserving therapy, lumpectomy followed by adjuvant whole breast irradiation (WBI) remains the standard of care. Randomized trials have demonstrated that the addition of a lumpectomy cavity boost significantly reduces the risk of ipsilateral breast tumor recurrences but also increases the risk of breast fibrosis. Contemporary randomized trials define the lumpectomy cavity boost volume as a 1.7 cm isometric expansion on the lumpectomy cavity as delineated on CT. However, identifying the lumpectomy cavity can be challenging, especially in women that receive adjuvant chemotherapy and in cases in which surgical clips are not present. Recently, the use of oncoplastic techniques in breast conserving surgery has increased. These techniques are used to prevent the poor cosmetic results that can occur when a large volume of breast tissue is resected. Women that undergo oncoplastic reconstruction represent especially difficult cases for lumpectomy cavity delineation. Retrospective series have evaluated the use of intraoperative electron radiotherapy (IOERT) as a boost prior to WBI in women receiving lumpectomy without oncoplastic reconstruction. In the largest series of IOERT boost prior to WBI the local control rate of this approach was >99%. Prospective data regarding IOERT boost in women undergoing oncoplastic reconstruction are limited. Advantages of this approach include direct visualization/irradiation of the tumor bed, sparing the skin of irradiation, and reducing the treatment time by ~1 week. We hypothesize that IOERT boost followed by WBI will result in acceptably low rates of grade 3 fibrosis in women undergoing lumpectomy with oncoplastic reconstruction.

Trial Design: This is a single-arm, prospective study to evaluate the safety, toxicity and efficacy of IOERT boost at the time of breast conserving surgery in women with early-stage breast cancer undergoing oncoplastic reconstruction. Eligible women will receive 1 dose of 8 Gy to the surgical bed after lumpectomy but prior to oncoplastic reconstruction. Women will then receive adjuvant WBI of 40 Gy in 15 fractions or 50 Gy in 25 fractions.

Eligibility: Key inclusion criteria include age ≥18 yo, clinically node-negative stage I/II, any breast cancer subtype.

Specific Aims: Our primary aim is to determine the rate of grade 3 breast fibrosis at 1 year. Additional aims include surgical complication rates, cosmesis, and local regional cancer control.

Statistical Methods: Safety will be evaluated by the rate of surgical complications necessitating hospital readmission or return to the operating room within 30 days of surgery+IOERT. If ≥4 events in the first 10 patients, ≥7 events in the first 20 patients, or ≥9 events in the first 30 patients are seen, the study will be halted. We hypothesize that the grade 3 fibrosis rate in our study will be ≤5%. Assuming an actual rate of 4%, an unacceptable rate of 9%, and a drop-out rate of 10%, the expected sample size is 176.

Patient Accrual: Current accrual is 0 of 176.

Contact Information: Sohyum McElroy (soyhun.mcelroy@osumc.edu) or Jose Bazan (jose.bazan2@osumc.edu)

Funding Source: Intraop Medical
**Title:** NRGI oncology/NSABP B-51/RTOG 1304: Phase III trial to determine if chest wall and regional nodal radiotherapy (CWRNRT) post mastectomy (Mx) or the addition of RNRT to breast RT post breast-conserving surgery (BCS) reduces invasive breast cancer recurrence-free interval (IBCR-FI) in patients (pts) with positive axillary (PAx) nodes who are ypN0 after neoadjuvant chemotherapy (NC)

Eleftherios P Mamounas\(^1,2\), Hanna Bandos\(^1,3\), Julia R White\(^4,5\), Thomas B Julian\(^1,6\), Atif J Khan\(^1,7\), Simona F Shaitelman\(^6,8\), Mylin A Torres\(^4,9\), Frank A Vicini\(^1,10\), Patricia A Ganz\(^1,11\), Susan A McCloskey\(^1,11\), Soonmyung Paik\(^1,12\), Nilendu Gupta\(^4,5\), X Allen Li\(^13\), Dominic J DiCostanzo\(^4,5\), Walter J Curran Jr\(^4,5\) and Norman Wolmark\(^1,6\). \(^1\)NRG Oncology/NSABP, Pittsburgh, PA; \(^2\)UF Health Cancer Center at Orlando Health, Orlando, FL; \(^3\)University of Pittsburgh, Pittsburgh, PA; \(^4\)NRG Oncology/RTOG, Philadelphia, PA; \(^5\)Ohio State University, Columbus, OH; \(^6\)Allegheny Health Network Cancer Institute, Pittsburgh, PA; \(^7\)Memorial Sloan Kettering Cancer Center, New York, NY; \(^8\)University of Texas MD Anderson Cancer Center, Houston, TX; \(^9\)Winship Cancer Institute Emory University, Atlanta, GA; \(^10\)21st Century Oncology (St. Joseph Mercy Hospital Campus), Pontiac, MI; \(^11\)University of California at Los Angeles, Los Angeles, CA; \(^12\)Yonsei University College of Medicine, Seoul, Korea and \(^13\)Medical College of Wisconsin, Milwaukee, WI.

**Body: Background:** This phase III post-NC trial evaluates if CWRNRT post-Mx or whole breast irradiation (WBI) with RNRT after BCS significantly reduces the IBCR-FI rate in pts with PAx nodes that are negative after NC. Secondary aims are OS, LRR-FI, DR-FI, DFS-DCIS, second primary cancer, and comparison of RT effect on cosmesis in reconstructed Mx pts. Correlative science examines RT effect by tumor subtype, molecular outcome predictors for residual disease pts, and predictors for the degree of reduction in locoregional recurrence. **Methods:** Clinical T1-3, N1 IBC PAx nodes (FNA or core needle biopsy) pts complete ≥8 weeks of NC (anthracycline and/or taxane). HER2+ pts receive anti-HER2 therapy. Following NC, BCS or Mx, sentinel node biopsy (≥2 nodes) and/or Ax dissection with histologically negative nodes is performed. ER/PR and HER-2neu status before NC is required. Pts receive required systemic therapy. Radiation credentialing with a facility questionnaire/case benchmark is required. Random assignment for Mx pts is to no CWRNRT or CWRNRT and for BCS pts to WBI or WBI+RNRT. **Statistics:** 1636 pts to be enrolled over 5 yrs (definitive analysis at 7.5 yrs). Study is powered at 80% to test that RT reduces the annual hazard rate of events for IBCR-FI by 35% for an absolute risk reduction of 4.6% (5-yr cumulative rate). Intent-to-treat analysis with 3 interim analyses (43, 86, and 129 events) and a 4th/final analysis at 172 events. Pt-reported outcomes focusing on RT effect will be provided by 736 pts before random assignment and at 3, 6, 12, and 24 mos. Accrual as of 6-12-17 is 645 (39.43%). **Contacts:** Protocol: CTSU member website https://www.ctsu.org. Questions: NRG Oncology Pgh Clin Coord Dpt: 1-800-477-7227 or ccd@nsabp.org. Pt entry: OPEN at https://open.ctsu.org or the OPEN tab on CTSU member website. **Support:** U10 CA-2166; -180868; -180822; 189867; Elekta.
Body: Background: The lumpectomy cavity (LPC) boost has been shown in 2 randomized studies to improve local control in breast cancer. Hypofraction is now being used for delivery of the LPC boost in some early-stage patients. This trial delivers the LPC boost in a single fraction using a novel breast immobilization device/treatment delivery system.

Trial design: Patients are enrolled in this trial after standard resection with lumpectomy/sentinel lymph node biopsy (as appropriate) and chemotherapy (as indicated per standard of care). At the time of CT simulation for whole-breast radiation therapy (RT), the radiation oncologist evaluates breast size and LPC position. If consented for treatment, the patient receives a single fraction “boost” treatment of 8 Gy in 1 fraction followed by standard whole-breast RT to start within 7 days of completion of the boost. Whole-breast radiation is delivered in the supine or prone position with the following fractionation schemes: 4005 cGy in 15 fractions or 5000 cGy in 25 fractions.

On the day of the boost treatment, the patient is fitted with the breast immobilization device, with a plastic inner cup that is fitted so that the breast fills all or most of the cup. A rigid outer cup with a built-in stereotactic fiducial system is attached. Moderate negative pressure is applied to immobilize the breast within the cup system. Patients then undergo CT simulation in the prone position. Clip placement and LPC cavity location must meet eligibility criteria before proceeding with treatment planning and delivery.

Eligibility criteria:
Eligibility criteria: age ≥60 yo; female only; dx of invasive ductal or lobular carcinoma or ductal carcinoma in situ; estrogen receptor positive; successful completion of lumpectomy ± sentinel lymph node biopsy with negative margins for invasive or noninvasive cancer; greatest tumor dimension <4 cm before surgery; weight <330 lb; height <76 inches; nonlactating and nonpregnant. Various additional dosimetric factors must be met prior to treatment. If these are unable to be met, the patient will become ineligible for treatment.

Specific aims: The aim of this study is to demonstrate the feasibility and safety of delivering the LPC boost RT using a single fraction with a novel immobilization device/treatment delivery system while ensuring coverage of the target volume with appropriate dose homogeneity and conformity. Secondary aims are evaluation of patient comfort, acute toxicity (1 month), and late toxicity (1 year).

Statistical methods: A Simon 2-stage design is utilized for this trial. After evaluating the device and treatment on 8 patients in the first stage, the trial was designed to be terminated and device rejected if the dose distribution was acceptable for ≤5 patients. The first stage was completed in spring 2017 and progressed to the second stage, designed to include a total of 17 patients.

Accrual and target accrual: Target accrual for this study is 14 patients successfully treated while meeting all protocol constraints. As of 6/2017, 16 patients have been enrolled, of whom 13 have been successfully treated while meeting all protocol constraints.
Title: Feasibility of assessing radiation response with MRI/CT directed preoperative accelerated partial breast irradiation in the prone position for hormone responsive early stage breast cancer

Julia White¹, Sohyun McElroy¹, Ashley Sekhon¹, Lai Wei², Jose Bazan¹, Xiangyu Yang³, Dominic DiCostanzo¹, Karla Kuhn¹, Nilendu Gupta¹ and Michael Knopp³. ¹Ohio State University, Columbus, OH; ²Ohio State University, Center for Biostatistics, Columbus, OH and ³Ohio State University, Columbus, OH.

Body: Background: Accelerated partial breast irradiation (APBI) delivers adjuvant radiation (RT) to the 1-2 cm of the breast at highest risk for recurrence surrounding the lumpectomy (L) cavity over 5-8 days and is an alternative to standard whole breast irradiation for hormone sensitive (HS) stage 1 (T1, N0) breast cancer (BC) based on 2 randomized controlled trials. External beam methods for APBI are common but have notable inherent drawbacks that include: inter-fraction inaccuracy due to patient setup based on anatomy, intra fraction error related to patient or respiratory motion, and inaccurate geometric targeting by relying on L cavity position instead of the tumor position. Postoperative RT has other limitations including delivery in the setting of disrupted blood or lymphatic supply that may be suboptimal for radio sensitivity and it eliminates observation of radiation-induced tumor response. MRI is an established tool for measuring BC extent and response from neoadjuvant systemic therapy. It’s hypothesized that MRI directed pre-operative APBI using intensity modulated radiotherapy (IMRT) with image guidance (IGRT) will improve RT delivery, and that MRI features can be identified to correlate with pathologic radiation response.

Trial Design: There are 2 cohorts to this single arm prospective trial. The first cohort is for establishing and verifying patient flow and image fusion between MRI, CT and RT planning. In the second cohort eligible patients will receive preoperative APBI 38.5 Gy in 10 fractions BID with IMRT, IGRT in the prone position using MRI defined targets fused to CT treatment planning.

Eligibility: For cohort 1 it is HS Stage 1 BC that has completed CT in prone position for RT planning. Eligibility for cohort 2 requires: age > 50 yo, clinical stage 1 BC, HS, HER2 negative, intending L, clinically negative axilla verified by ultrasound, able to tolerate the prone position, and MRI with contrast.

Specific aims: To determine the reproducibility of MRI directed preoperative APBI based on meeting 3 criteria: ability to define RT targets by MRI, quality of RT plans and completion of treatment (APBI and surgery). Additional aims include assessing toxicity, cosmetic outcome, local regional cancer control and collection of tissue for correlative studies.

Statistical methods: The optimal two-stage design by Simon is used. Sample size for cohort 2 is based on the first endpoint. 19 eligible patients will be required in the first stage; if 3 or more treatments are scored unacceptable, then early stopping will be recommended. Otherwise, accrual will continue to a total accrual of 30. If > 4 of 30 treatments are scored unacceptable, the technique will be considered not reproducible, and a Phase II study will not be pursued. Under the null hypothesis of an 80% reproducibility rate, this two-stage design has an expected sample size of 24.4.

Patient accrual to cohort 1 has completed the targeted accrual of 3. Patient accrual to Cohort 2 is 5/30.

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Funding source: Susan G Komen Breast Cancer Foundation Grant # GRT00035216
Title: An open label, phase II trial of continuous low-irradiance photodynamic therapy (CLIPT) using verteporfin for the treatment of cutaneous breast cancer metastases

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Body: Background
Cutaneous metastases occur in approximately 20% of patients (pts) with metastatic breast cancer (mBC) and can be highly symptomatic and distressing. Radiation therapy is frequently used, but progression often occurs quickly. Systemic therapies are also typically used, but also often result in limited benefit. Photodynamic therapy is a promising approach with encouraging results in small studies. Here we will evaluate a novel Continuous Low-Irradiance Photodynamic Therapy (CLIPT) system that emits 690nm LED via a handheld Power Pack attached to a single-use sterile Light Patch to deliver a total energy level of 20J/cm². Verteporfin (Visudyne®) is a photosensitizer approved for ophthalmological use that, when combined with CLIPT, generates activated oxygen species which can destroy tumor cells with limited normal tissue reaction.

Methods
This open label, Phase 2 study will evaluate the efficacy and safety of CLIPT with verteporfin in 15 patients with cutaneous lesions from mBC. Patients will receive a single IV injection of Verteporfin on day 1. The 9x9cm Light Patch with an adhesive border is placed over the treatment site and attached to the CLIPT portable Power Pack. The patient turns the device on at home 6 hours after the Verteporfin injection and it automatically turns off after 24 hours. The patient then removes the Light Patch and returns to clinic on day 3. The primary endpoint is objective response rate (RR) at 3 weeks following CLIPT using a modified RECIST which accounts for nodular or diffuse plaque-like lesions. Response will be confirmed by independent dermatologist review. Secondary endpoints include RR at 2, 8 and 12 weeks, toxicity, and quality of life (using FACT-B and Brief Pain Inventory). A novel Participant Symptom Scale (PSS) will also be used in which the first 8 patients will list their most distressing symptoms from cutaneous metastases and score the severity of the symptoms from 1 to 10. The six most common symptoms among the first 8 patients will then be used in the PSS for the remaining 7 patients. The PSS will be assessed at baseline and at subsequent visits to explore any improvement in severity of symptoms after treatment with CLIPT. Patients who derive clinical benefit may be retreated up to 3 times to the same or different region. Eligible patients will have: cutaneous metastases from mBC with measurable disease by protocol defined modified RECIST 1.1, ≥ 1 line of prior systemic or local therapy for mBC, ≥ 14 days from prior systemic therapy or 60 days from radiation to target lesion, and no expectation for systemic therapy for ≥ 14 days after CLIPT. RR will be reported with 95% CI. With 15 patients, if ≥ 3 responses (RR ≥ 20%) are observed, the null hypothesis of RR ≤ 5% will be rejected. At the time of abstract submission, 4 patients have been accrued. Clinical Trials Reg: NCT02939274
**Title:** A phase II randomized trial of gemcitabine plus cisplatin (GP) vs. gemcitabine plus carboplatin (GC) as the first-line treatment for patients with metastatic triple negative breast cancer

Chengcheng Gong¹, Enying Cao¹, Zhonghua Wang¹, Jian Zhang¹, Leiping Wang¹, Jun Cao¹, Sheng Zhang¹, Zhonghua Tao¹, Ting Li¹, Yannan Zhao¹, Yi Li¹, Biyun Wang¹ and Xichun Hu¹. ¹Fudan University Shanghai Cancer Center, Shanghai, China.

**Body:** Background:
Triple-negative breast cancer (TNBC) is an aggressive disease with limited treatment options and poor prognosis. Gemcitabine plus carboplatin (GC) is one of the preferred chemotherapeutic regimens for patients with metastatic triple-negative breast cancer (mTNBC), with a median progression-free survival (PFS) of 4.6 months in the first-line patients (O'Shaughnessy J, et al. J Clin Oncol 2014). Gemcitabine plus cisplatin (GP) has also demonstrated promising efficacy and safety in the first-line phase III trial of mTNBC, with a median PFS of 7.7 months (Hu XC, Lancet Oncol 2015). A recent analysis, based on data derived from a cohort of 379 mTNBC patients, indicated that patients receiving cisplatin-based regimen as the first-line chemotherapy showed better PFS compared with other platinum agents (8.0 vs 4.3 months, P = 0.03) (Zhang J, et al. Oncotarget 2015). To further investigate the superiority between carboplatin and cisplatin when combined with gemcitabine, this phase II study was conducted to directly compare the efficacy and safety of GP with GC in the first-line treatment for patients with metastatic triple negative breast cancer.

**Trial Design:**
This prospective phase II, single center, open-label, randomized study has been designed to compare the efficacy and safety of GP with GC as the first-line treatment for mTNBC. Patients are randomized 1:1 to receive gemcitabine (1250 mg/m², D1,8) plus cisplatin (75 mg/m², D1) or gemcitabine (1000mg/m², D1,8) plus carboplatin (area under the curve 2 mg × min/mL, D1,8) every 21 days until disease progression or intolerable toxicity.

**Eligibility Criteria:**
Patients with histologically confirmed triple negative metastatic breast cancer, with no prior chemotherapy in metastatic setting will be included in this trial. Eligible patients must be between 18 and 70 years of age with a performance status of 0–1, adequate organ function and at least one RECIST 1.1-measurable lesion.

**Specific Aims:**
The primary endpoint is PFS. Secondary endpoints include objective response rate, safety and overall survival.

**Statistical Methods:**
The sample size of the present study was determined based on the results of two phase III clinical trials: the median PFS for patients receiving GC and GP as the first-line treatment for mTNBC was 4.6 and 7.7 months, respectively. This design was hypothesized that GP would be superior to GC in terms of efficacy. Thus, in order to detect an improvement of median PFS from 4.6 months to 7.7 months, with 80% power and a 1-sided type I error of 0.05, 136 patients would be required. Considering a drop-out rate of 10%, a total of 150 patients planned to be enrolled.

**Present Accrual and Target Accrual:**

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Biyun Wang, MD pro_wangbiyun@163.com
ClinicalTrials.gov Identifier: NCT02341911.
Title: A phase II study of metronomic daily oral vinorelbine as first-line chemotherapy in advanced/metastatic hormone receptor positive (HR+) / human epidermal growth factor receptor 2 negative (HER2-) breast cancer resistant to endocrine therapy - VinoMetro

Marcus Schmidt¹, Thomas Decker², Tanja Fehm³, Stefan Fuxius⁴, Nadia Harbeck⁵, Ingolf Juhasz-Böss⁶, Christoph Thomssen⁷, Martina Seehase¹, Lukas Schollenberger⁸, Christian Ruckes⁹ and Volker Möbus⁹. ¹Klinik und Poliklinik für Geburtshilfe und Frauengesundheit, Universitätsmedizin Mainz, Mainz, Germany; ²Schwerpunktpraxis für Hämatologie und Onkologie, Ravensburg, Germany; ³Frauenklinik, Universitätsklinikum Düsseldorf, Düsseldorf, Germany; ⁴Onkologische Schwerpunktpraxis, Heidelberg, Germany; ⁵Brustzentrum, Klinikum der Ludwig-Maximilians-Universität, Munich, Germany; ⁶Klinik für Frauenheilkunde, Geburtshilfe und Reproduktionsmedizin, Universitätsklinikum des Saarlandes, Homburg, Germany; ⁷Klinik und Poliklinik für Gynäkologie, Universitätsklinikum Halle, Halle (Saale), Germany; ⁸Interdisziplinäres Zentrum Klinische Studien (IZKS), Universitätsmedizin Mainz, Mainz, Germany and ⁹Klinik für Gynäkologie und Geburtshilfe, Klinikum Frankfurt Höchst, Frankfurt, Germany.

Body: Background
Chemotherapy (CTx) is a cornerstone in HR+/HER2- advanced/metastatic breast cancer (a/mBC) after failure of endocrine treatment. In this indication, vinorelbine (VRL) is a well-established cytotoxic drug. There is a high medical need for new options that prolong the time between endocrine failure and intensive CTx, which is commonly associated with impaired quality of life and serious side effects. Metronomic CTx was shown to induce disease control in a/mBC with a favorable safety profile. This innovative approach involving continuous daily dosing of oral VRL, which could provide anti-angiogenic and immune-modulatory properties, has not been investigated so far in this indication.

Trial Design
VinoMetro is an open-label, single-arm, phase II study (Simon two-stage minimax) of metronomic daily oral VRL (30 mg/day) as first-line CTx. The study involves strict safety monitoring with an initial safety run-in. It is accompanied by a steering committee and supervised by an independent data safety and monitoring board (DSMB). The main objectives are to estimate efficacy in terms of clinical benefit rate after 24 weeks of treatment (primary endpoint) and the progression-free survival, amongst others, as well as the assessment of safety and quality of life. Patients with HR+/HER2- a/mBC having failed or being no candidate for endocrine therapy (targeted combinations allowed) and being naïve to palliative CTx are eligible, if they exhibit ECOG 0-1. The main exclusion criteria are prior vinca-alkaloids, aggressive disease requiring combination CTx and CNS involvement.

Until 2017-05-31, 7 patients were enrolled. It is planned to include 45 (39 evaluable) patients at 8 German sites until 09/2018. Scheduled completion date is 09/2019. Two interim analyses are planned (first analysis: safety evaluation based on the 10 initial patients with predefined stopping rules). Depending on recruitment, it is planned to include the interim safety data in the congress presentation.

VinoMetro is an investigator initiated trial (NCT03007992) sponsored by the University Medical Centre of Johannes Gutenberg-University Mainz, Germany, and supported by an unrestricted grant provided by Pierre Fabre Pharma GmbH (Freiburg, Germany).
Does minocycline mitigate chemotherapy induced neuroinflammation? A phase II randomized placebo controlled study

Hinda Boutrid1, Raquel Reinbolt1, Michael Knopp2, Nicole Williams1, Jeffrey VanDeusen1, Sagar Sardesai1, Anne Noonan1, Laura Flora3, Erica Gleich3, Xueliang Pan4, Michael Berger5, Craig Vargo5, Robert Wesolowski1, Bhuvaneswari Ramaswamy1, Anne C DeVries6 and Maryam Lustberg1.  
1The Ohio State University Wexner Medical Center, Columbus, OH; 2The Ohio State University Wexner Medical Center, Columbus, OH; 3The Ohio State Comprehensive Cancer Center Clinical Trials Office, Columbus, OH; 4The Ohio State University, Columbus, OH; 5Stefanie Spielman Comprehensive Breast Center, Columbus, OH and 6The Ohio State Wexner Medical Center, Columbus, OH.

Body: Background: Many breast cancer (BC) patients, particularly those who receive chemotherapy (chemo), experience affective symptoms and cognitive changes that can negatively impact their quality of life. Causal links between inflammatory mediators and the development of depressive-like behavior and cognitive defects, have been established in mouse models, including studies by our group showing increased microglial activation following chemo (A.C DeVries et al). Microglia are resident immune cells of the brain, which release proinflammatory cytokines when activated. Doxorubicin (DOX) induces microglial activation in the brain. Minocycline, a second generation tetracycline, has been shown to suppress inflammation by inhibiting microglial activation in CNS disease models. We hypothesize that (1) chemo activates microglia in the brains of women being treated for BC, which can precipitate or exacerbate depression, anxiety and cognitive deficits and (2) Minocycline administration during neoadjuvant or adjuvant chemo will prevent chemo-induced microglial activation and will reduce affective and cognitive symptom burden. 

Trial Design: This is a single center, Phase II, double blinded randomized study of minocycline (100 mg twice a day) vs placebo twice a day in women with BC receiving DOX-based or other chemo for BC. Pts will be randomized to either oral minocycline or placebo for up to a 1 week loading period plus chemo treatment period and an optional subsequent 2 week period. 

Eligibility Criteria: Women diagnosed with BC stages I-III initiating first line adjuvant or neoadjuvant chemo. 

Aims: (1) to evaluate symptoms related to anxiety and depression and cognitive changes during and after chemo completion (2) to evaluate markers of neuro inflammation as assessed by blood based inflammatory cytokines and central markers of inflammation and microglia activation using 18F-Fludeoxyglucose and 11C-PK11195 positron emission tomography. Primary endpoints are changes in Center for Epidemiological Studies Depression Scale (CES-D) and State Trait Anxiety Index (STAI) from baseline to end of study after minocycline vs placebo intervention. Secondary endpoints are changes in cognitive function during chemo using validated cognitive testing including N-Back Test, Behavioural Rating Inventory of Executive Function (BRIEF) and the Multifactorial Memory Questionnaire Ability Scale (MMQ). 

Statistical Methods: Primary analysis for efficacy will be intention-to-treat. The main objective is to preliminarily evaluate the effect of minocycline on chemo-induced depressive symptoms in terms of changes in CES-D and STAI scores. Mixed models will be used to evaluate cognitive function changes. A sample size of 23 per group, will give 80% power to detect an effect size of 0.74 standard deviation (SD) difference between the 2 groups at significance level of 0.10 based on a 2 sided two-sample t-test. From our experience, attrition of less than 20% is expected for studies in this patient population in our center, and to account for this, we plan to recruit up to 60 patients. 

Contact: Study PI: Maryam.lustberg@osumc.edu
Title: Phase I/II study of T-DM1 alone versus T-DM1 and metronomic temozolomide in secondary prevention of HER2-Positive breast cancer brain metastases following stereotactic radiosurgery

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Background: Brain metastases occur in up to 25-40% of HER2+ breast cancer patients. Standard treatment is limited to surgery or stereotactic radiosurgery (SRS) and/or whole brain radiation therapy (WBRT), with high levels of recurrence or progression, limiting survival and quality of life in most patients. Our group has demonstrated that low doses of temozolomide (TMZ) administered in a prophylactic, metronomic fashion can significantly prevent development of brain metastases in murine models of breast cancer. Based on these findings, we propose a secondary-prevention clinical trial.

Trial Design: Phase I/II open label study. Phase I will follow a standard 3+3 design: T-DM1 3.6 mg/kg IV every 21 days plus TMZ 30, 40 or 50 mg/m² daily. Phase II: randomization T-DM1 3.6 mg/kg versus T-DM1 3.6mg/kg plus TMZ at recommended phase 2 dose (RP2D). Patients will undergo radiology guided lumbar puncture at baseline and after 6 weeks of treatment (C3D1) for correlative studies, brain MRI, systemic restaging CTs, and questionnaires for evaluation of symptoms and quality of life (MDASI-BT and PROMIS®) every 6 weeks.

Eligibility: HER2+ breast cancer with ≤3 brain metastases, treated with SRS and/or resection ≤6 weeks before enrollment, no leptomeningeal metastases, no previous WBRT, able to complete brain MRI with contrast evaluations, willing to undergo lumbar puncture, ECOG ≤2 and adequate organ and marrow function. HBV, HCV or HIV-positive patients are ineligible.

Specific Aims: Phase I: to identify the maximum tolerated dose (MTD) of TMZ combined with T-DM1. Phase II: to determine if the combination regimen of T-DM1 and TMZ improves the recurrence-free incidence from distant new brain metastases at one year as compared to T-DM1 alone. Biomarkers, including cell free DNA sequencing from CSF, serum and tumor block, serum markers for neuroinflammation, and patient reported outcomes, will be analyzed in an exploratory fashion.

Statistical Methods: Phase I, MTD will be identified based on the dose level at which 0 or 1 patient in 6 has a DLT. Phase II, to test whether TMZ will increase RFS from 50% to 65% at 12 months. RFS Kaplan-Meier curves will be created for each of the randomized arms and compared using a one-tailed log-rank test, with a one-sided 0.10 significance level of interest to be detected. Patients will be stratified for number of brain lesions and status of systemic metastases (controlled or not).

Target Accrual: 49 evaluable patients per arm (total 98), plus 9 to 18 patients during phase I. Trial will open in Summer 2017, at NIH in Bethesda, MD.

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Title: A randomized phase II study of maintenance hormone therapy with or without capecitabine after induction therapy with bevacizumab plus paclitaxel in hormone receptor positive and HER2 negative metastatic breast cancer (KBCSG-TR1214)

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Body: Background: The combination therapy of Bevacizumab (B) and Paclitaxel (P) has proved to prolong progression free survival (PFS) in E2100 and MERiDiAN study for advanced and metastatic breast cancer(AMBC). Because of its longer PFS, developing optimal therapeutic strategy of B+P to improve survival, including management of toxicity is crucial. From the International Consensus Conference for Advanced Breast Cancer, most experts agreed the maintenance endocrine therapy after effective induction chemotherapy in AMBC. In KBCSG-TR 1214 study, we planned to examine the following clinical questions. 1. As a maintenance therapy, which is more effective either endocrine therapy alone (E) or endocrine therapy with capecitabine (E+C)? 2. Can maintenance therapy reduce toxicity of B+P and restore patient's QOL.? 3. How effective is B+P re-challenge after failure of maintenance therapy?

Methods: KBCSG-TR 1214 study is multicenter open-labeled randomized phase II trial for hormone receptor (HR)-positive and HER2-nagative patientswho have experienced none or one prior chemotherapy for AMBC. Patients will receive B (10mg/kg q2w) in combination with P (90mg/m² on day 1, 8, and 15 q4w) as an induction therapy. Patients without progression after 6 cycles of B+P will be randomized to E or E+C. Endocrine treatment has been administrated by their physician's choice. Patients in E+C will receive endocrine therapy with capecitabine 1657mg/m² on day1 to 21 q4w. Stratification factors for randomization are menopausal status, presence of target lesion, number of prior endocrine therapies for AMBC, with or without 1st line chemotherapy for AMBC. After progression of maintenance therapy (E or E+C), B+P will be started again as a re-challenge therapy. Primary end point is PFS of maintenance therapy. Secondary end points include time to failure of strategy from randomization, efficacy of re-challenge therapy, overall survival and safety of induction therapy. Translational research is also planned. VEGF, angiopoetin-1, and apelin in plasma will be measured at four points (before induction therapy, at the beginning of the maintenance therapy and the re-induction therapy, and at the end of the trial). The sample size was calculated by typeIerror (1-sided) of 0.05 and 80% power to estimate median PFS of each maintenance therapy 9 months with a threshold of 6 months. The target number of patients enrolled and randomized after induction therapy was 120 and 90, respectively. Enrollment has been completed with 116 patients as of April, 2016 and 90 patients had been successful to shift to the maintenance phase with randomization. The last patient had been randomized on October, 2016. The first analysis will be planned during the second quarter of 2018 (UMIN000008662).
Title: METAMORPH: METAstatic markers of recurrent tumor PHenotype for breast cancer

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Body: Up to 30% of patients diagnosed with breast cancer will develop recurrent disease within their lifetime, and currently this form of the disease is incurable. There are unmet needs to better understand underlying metastatic biology, identify new therapeutic targets and develop better methods for monitoring changes in disease, both to monitor response and elucidate resistance mechanisms. To address these needs, the METAMORPH Study encompasses a comprehensive approach that combines serial molecular tissue profiling at the RNA and DNA level with circulating markers (DTCs, CTCs, plasma tumor DNA), and ongoing assessment of therapeutic response.

METAMORPH is a prospective cohort study of women with suspected or confirmed recurrent breast cancer and accessible tumor by standard clinical biopsy, who are enrolled at the University of Pennsylvania prior to starting a new therapy for recurrent metastatic disease. The aims of this trial are to (1) evaluate the mechanisms through which recurrent breast cancer are genetically distinct from the primary tumor, (2) evaluate the circulating tumor biomarker trajectory of recurrent disease, (3) elucidate “escape pathways” of progressing tumors that emerge during the selective pressure of therapy, and (4) explore clinical utility of tumor and blood testing. The study protocol integrates research aims into clinical care, including a standardized approach to disease assessment and biopsy, pathologic confirmation of histology and receptor subtype, panel-based CLIA-approved genomic profiling, collection of research specimens, and standardized reporting of results, which are returned to patients and physicians. Patients are followed for treatment and outcome, and serial samples are collected at progression. A companion protocol, COMET, provides education about genomic testing and assesses patient understanding and impact of results. To date, 155 patients have enrolled, 142 (92%) have been biopsied, 120 (77%) have had sufficient DNA for molecular profiling and 109 (70%) have had genomic panel testing. Accrual is ongoing, with an initial target of 300 patients. Multiple sites within the UPHS Health System are enrolling. Contact information: angela.demichele@uphs.upenn.edu.

Key words: Metastatic disease, tumor profiling.
Title: Phase Ib study of rebastinib plus antitubulin therapy with paclitaxel or eribulin in patients with metastatic breast cancer

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Body: Background: Metastasis is the primary cause of death in breast cancer, yet no specific therapies are available that inhibit the metastatic process. TMEM (Tumor Microenvironment of Metastasis) are microanatomic structures formed by a Mena-expressing tumor cell, Tie2-expressing macrophage, and endothelial cell in direct content, which serve as the primary portal for tumor cell intravasation into the circulation and subsequent metastasis. High TMEM score in the primary tumor is associated with higher risk of recurrence in ER+, HER2- early breast cancer. Paclitaxel induces the formation of TMEM in the primary tumors of patients treated with neoadjuvant chemotherapy, and in the primary tumor and distant metastases in the PyMT/PDX models. Tumor cell intravasation is mediated by release of VEGF that promotes focal vascular leakiness specifically at TMEM sites, and is derived from TMEM-associated Tie2HI/VEGFHI macrophages that release VEGF upon binding of the Tie2 receptor to angiopoietin2 (ANG2), which is elaborated by TMEM-associated endothelial cells. Moreover, ANG2-stimulated release of IL-10 by tumor-associated macrophages suppresses T cell proliferation, increases the ratio of CD4+ T cells to CD8+ T cells, and promotes the expansion of CD4+CD25highFOXP3+ cells. The Tie2 inhibitor rebastinib inhibits intravasation at TMEM sites, reduces circulating tumor cell (CTC) burden, prevents distant metastases, and improves survival in breast cancer animal models when added to either paclitaxel or eribulin. We therefore hypothesize that the addition of a potent Tie2 inhibitor (rebastinib) to antitubulin therapy in patients with HER2 negative metastatic breast cancer (MBC) will prevent hematogenous dissemination and distant metastasis by inhibition of TMEM function, reduction in CTC burden, and inhibition of immune-system suppression resulting in improvement in breast clinical outcomes.

Methods: Primary objective of this phase Ib study (NCT02824575) is to evaluate safety and tolerability of rebastinib in two dose levels (DL) (50mg or 100mg po BID) combined with paclitaxel IV 80mg/m² (day 1, 8 and 15) or eribulin IV 1.4mg/m² (day 1 and 8) for four 21-day cycles.

Key eligibility includes histologically confirmed HER2 negative MBC. ≤ 2 non-taxane chemotherapy regimens are allowed for rebastinib plus paclitaxel arm, while ≥ 2 chemotherapy regimens (including a taxane) are required for eribulin plus rebastinib arm. ≥ 2 endocrine regimens, including an approved CDK4/6 inhibitor, is required for ER+ disease. Patients require ECOG PS 0 or 1 and normal organ and marrow function. Exclusion criteria include significant ocular disease, significant history of cardiac disease or concomitant use drugs that prolong QTc interval.

Pharmacodynamic biomarkers to be measured during cycle 1-3 include CTCs, ANG 1/2 levels and Tie-2 expressing monocytes. Tissue biopsy after two treatment cycles in 6 patients who have accessible tumors will be performed to evaluate TMEM score and function. With two DL of rebastinib, and 3-6 patients at each DL, it is anticipated that 6-12 patients will be required. This trial has enrolled three patients assigned paclitaxel arm (DL1) and one patient in eribulin arm (DL1).
Title: Phase 1/2 trial of the oncolytic virus, talimogene laherparpvec, in combination with neoadjuvant chemotherapy in stage II/III triple negative breast cancer

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Background: The host anti-tumor immune response plays an important role in determining natural history and therapy response for early stage breast cancer. Tumors with high levels of lymphocytic infiltration appear to have a superior prognosis and response rate to neoadjuvant chemotherapy. However, these tumors are in the minority so methods to enhance tumor lymphocyte infiltration should be identified. The oncolytic virus, talimogene laherparpvec (TVEC) is a genetically modified HSV1 virus which selectively replicates in transformed cells while sparing normal tissue. This leads to lysis of infected tumor cells along with co-expression of GM-CSF to elicit an enhanced anti-tumor immune response. Prior data has shown TVEC can be safely combined with chemotherapy in other indications, so we launched an investigator initiated study to determine the safety and efficacy of combining TVEC with neoadjuvant dose dense chemotherapy in stage II-III TNBC.

Study design: The study is a phase 1 (2 dose levels of TVEC, 3+3 design) and phase 2 single arm Simon two stage combination trial. Primary endpoints of phase 1 is safety of intratumoral TVEC (DL1=10⁶ PFU x 5 injections, DL2=10⁶ PFU x 1 then 10⁸ PFU x 4 injections) administered q2-3 weeks concurrently with weekly paclitaxel followed by standard dose dense AC x 4 and local therapy as indicated. Phase 2 primary endpoint is pCR rate of the study treatment, secondary endpoints include DFS, OS, immune correlates in resected tumor specimens. Eligibility criteria includes females >17 years old, newly diagnosed T2-3N0-3 TNBC, adequate organ function, primary tumor amenable to injection with TVEC, no immunosuppressive or autoimmune conditions, no inflammatory or bilateral/multifocal disease. Sample size is up to 49 patients (12 phase 1, 37 phase 2) with 80% power to detect increase in pCR rate from 30% to 50% with one sided p=.1 in phase 2.

Study status: This novel Amgen supported investigator initiated study activated to accrual 3/2017 and first patient on study was on 5/2017. The study is currently open only at the Moffitt Cancer Center. Target study completion date 8/2021. (NCT02779855)
Body: **Background:** Antiangiogenic agents have shown activity in breast cancer; however their use should be optimized. One potential solution is designing rational combinations based on blocking specific mechanisms of resistance. We previously published that tumors exposed to bevacizumab (BEV) can either experience vascular pruning (followed by increased tumor hypoxia) or vascular normalization (followed by a switch to mitochondrial metabolism). FDG-PET can monitor which pattern is occurring as early as 8 days after the first dose (Mol Oncol; 10: 704-18). We have also shown that when vascular normalization occurs, tumors become highly sensitive to mitochondrial inhibitors (Cell Rep 2016; 15: 1-14). ME-344 is a mitochondrial respiration inhibitor that has recently completed phase I. We sought to test the pharmacodynamic effects of ME-344 and its effects in Ki67 and cleaved caspase 3 (CC3) after single-dose BEV in treatment-naive early HER2-negative breast cancer patients, and whether the effects are restricted or not to those patients experiencing vascular normalization as predicted by FDG-PET.

**Trial design**
Placebo-controlled, two-arm, randomized, multicentric phase 0 trial. Treatment-naive HER2-negative breast cancer patients undergo a breast FDG-PET and a baseline tumor biopsy (frozen and paraffinized cores), followed by a single (15 mg/kg) BEV dose. FDG-PET is repeated on day +8. Patients are then randomized to three ME-344 (10 mg/kg) doses or placebo (days +8, +15 and +22). A second biopsy is then harvested, ending trial procedures. Patients can then follow surgery or neoadjuvant treatment according to their physician's criterion. Serial paraffinized biopsies are used to measure Ki67 and CC3; frozen biopsies are used for measuring mitochondrial activity with SDH enzymo-histochemistry (EHC) and assessing vessel architecture. Toxicity is graded with NCI CTC AE V4.03 criteria.

**Eligibility criteria**
1) Women >18 year old; 2) diagnosed of HER2-negative breast cancer; 3) patients must be treatment-naive; 4) ECOG 0/1; 5) lack of neuropathy; 6) primary tumor > 1cm.

**Specific aims**

**Primary:**
1) To study the effects in cell replication and cell death as a surrogate measure of efficacy of ME-344 added to BEV in treatment-naive breast cancer.
2) To determine if FDG-PET accurately detects patients experiencing vascular normalization early after BEV dosing.
3) To observe the effects of ME-344 in mitochondrial activity at the tumor level by performing SDH EHC.
4) To assess if ME-344 efficacy (measured by changes in CC3 and Ki67) is restricted or not to those patients experiencing vascular normalization and mitochondrial switch.

**Secondary:**
5) To study the tolerability of the combination of BEV plus ME-344.

**Statistical methods**
The sample size is calculated on the basis of the expected change in Ki-67 staining in the primary tumors in patients receiving ME-344 (-10% on average). With an $\alpha$ and $\beta$ errors of 5% and 20% respectively, the minimum number of patients necessary to observe a 10% decrease is 20 per arm. Changes in CC3, Ki67, SDH activity and FDG-PET SUV will be compared with intra-subject measurements and Z-scores.

**Accrual:** 13 of 40 planned patients.

**Contact:** acsalgado86@gmail.com
IMpassion031: A phase III study comparing neoadjuvant atezolizumab vs placebo in combination with nab-paclitaxel–based chemotherapy in early triple-negative breast cancer (TNBC)

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Body: Background Atezolizumab is an anti–programmed death-ligand 1 (PD-L1) monoclonal antibody that blocks the binding of PD-L1 to PD-1 and B7.1 receptors, thereby restoring tumor-specific immunity. TNBC is characterized by PD-L1 expression on tumor-infiltrating immune cells (IC), high levels of tumor-infiltrating lymphocytes (TILs), and a higher mutation rate compared with other breast cancers suggesting a therapeutic opportunity for atezolizumab. Atezolizumab alone and in combination with nab-paclitaxel is well tolerated, with promising clinical activity in metastatic TNBC. Furthermore, cytotoxic chemotherapies like nab-paclitaxel can enhance anti-tumor immune responses via neoantigen release. Taken together, this supports the investigation of atezolizumab in combination with nab-paclitaxel in early-stage TNBC. IMpassion031 is a global Phase III, double-blind, randomized, multicenter, placebo-controlled study being conducted to evaluate the efficacy and safety of neoadjuvant treatment with nab-paclitaxel → doxorubicin + cyclophosphamide and either atezolizumab or placebo in invasive stage II/III early TNBC. The selection and sequence of chemotherapy has been chosen to maximize the opportunity for a robust immune response.

Methods: Eligible patients are those with previously untreated, central laboratory–confirmed invasive TNBC with primary tumor size > 2 cm and ECOG PS 0-1. Exclusion criteria include history of invasive breast cancer, stage IV disease, bilateral breast cancer, prior systemic therapy for the treatment or prevention of breast cancer, prior immunotherapy and a history of autoimmune disease. Approximately 204 patients will be randomized 1:1 to receive atezolizumab (840 mg q2w) or placebo with nab-paclitaxel (125 mg/m² qw) for 12 weeks. Subsequent atezolizumab (840 mg q2w) or placebo with doxorubicin (60 mg/m² q2w) + cyclophosphamide (600 mg/m² q2w) will be given for 4 cycles prior to surgery. Post-surgery, patients will be unblinded. Patients in the atezolizumab arm will continue to receive atezolizumab (1200 mg q3w × 11 doses) post-surgery. Stratification factors include stage II vs III TNBC at diagnosis and PD-L1 expression on tumor-infiltrating IC (IC0 < 1% vs IC1/2/3 ≥ 1% with the VENTANA SP142 IHC assay). The primary endpoint is pathologic complete response (pCR); key secondary endpoints include pCR according to PD-L1 IC status, patient-reported outcomes, event-free survival and overall survival. Tumor samples will be taken at baseline, on treatment (optional), at surgery and post-recurrence for the assessment of biomarkers associated with treatment response and immune escape. (NCT pending).
Body: Background: Advanced breast cancer (BC) and endometrial cancer (EC) have limited treatment options with no treatments improving survival. ONC201 is the founding member of a novel class of anticancer drugs called imipridones. The drug is orally bioavailable and crosses the blood brain barrier. Preclinical studies have demonstrated that ONC201 selectively kills various cancer cells, including all subtypes of BC and EC, while having little effect on normal cells. An on-going Phase 1 study of ONC201 has demonstrated clinical benefit in some solid tumors, including EC and glioblastomas.

Trial Design: Phase 2 single arm study of ONC201 with 3 cohorts: Cohort 1, female and male hormone receptor positive breast cancer (HR+BC); Cohort 2, female and male triple negative breast cancer (TNBC); and Cohort 3, EC. All patients will receive ONC201 at the recommended Phase 2 dose of 625mg by mouth q7 days (1 cycle = 28 days). Patients will undergo a baseline biopsy as well as a biopsy after 5 doses of ONC201 (C2D2). Patients will be evaluated for response every two cycles (8 weeks) by RECIST 1.1.

Eligibility Criteria: Measurable disease with ≥1 biopsiable lesion, willing to undergo biopsies. Cohort 1 (HR+BC) requires prior treatment with >2 lines of hormonal treatment. No prior treatment required for the other cohorts. Patients must have ECOG 0-1 and adequate organ function. Patients with asymptomatic or brain metastases treated > 4 weeks from study entry are eligible. Exclusion criteria include: symptomatic CNS metastases, radiotherapy ≤4 weeks from study entry, HIV, Hepatitis B or Hepatitis C.

Specific Aims: Primary objectives for this study are progression free survival (PFS) at 8 months for Cohort 1 (HR+BC) and overall response rate (ORR) for Cohorts 2 and 3 (TNBC and EC). Secondary objectives include safety, clinical benefit rate (CBR = partial response + complete response + stable disease), and overall survival.

Statistical Methods: This study has been designed to pause prior to full accrual to allow for evaluation of futility prior to proceeding to full accrual. In Cohort 1, if ≥1 of 5 patients is progression-free at 8 months, then we will recruit up to 24 patients. In Cohort 2, if ≥2 of 10 patients has clinical benefit then we will recruit up to 29 patients. For Cohort 3, if 1 of 13 patients has clinical benefit, then we will recruit up to 25 patients. Additional evaluations of tumor or blood samples performed will be done in an exploratory fashion, with results presented without any formal adjustment for multiple comparisons.

Target Accrual: 24 patients with HR+BC, 29 patients with TNBC, and 25 patients with EC. This trial will open Summer 2017 at the National Institutes of Health (Bethesda, MD).

Contact Information: Principal Investigator Alexandra S Zimmer, MD; alexandra.zimmer@nih.gov
Title: A phase III, randomized trial of sacituzumab govitecan (IMMU-132) vs treatment of physician choice (TPC) for metastatic triple-negative breast cancer (mTNBC)

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Body: Background: Metastatic TNBC has an aggressive course with limited therapy options and poor survival. Sacituzumab govitecan (IMMU-132) is a novel antibody drug conjugate consisting of SN-38, the active metabolite of the topoisomerase I inhibitor, irinotecan, conjugated to a humanized mAb targeting Trop-2, which is highly expressed in most epithelial cancers, including TNBC. We previously reported that patients (pts) with mTNBC treated with IMMU-132 after a median of 5 prior therapies from initial diagnosis achieved a 30% objective response rate (ORR), 8.9 mo median duration of response (DOR), and an acceptable safety profile with nausea, neutropenia, and diarrhea the most common toxicities (Bardia et al., JCO, 2017). IMMU-132 was awarded Breakthrough Designation by the FDA based on this data. Accordingly, we are enrolling additional patients with relapsed/refractory mTNBC with intention of seeking regulatory approval as a ≥3rd-line therapeutic option.

Trial design: An international, open-label, Phase III study in pts with refractory/relapsed mTNBC after ≥2 prior chemotherapies for advanced disease or >1 therapy for pts who progress within 12 months of adjuvant therapy (NCT02574455). Pts are randomized 1:1 to receive either IMMU-132 (10 mg/kg IV, days 1 and 8 every 21 days) or TPC from one of 4 prespecified single-agent regimens (capecitabine, eribulin, vinorelbine or gemcitabine). Pts continue treatment until progression requiring discontinuation or unacceptable toxicity. The primary endpoint is progression-free survival (PFS) and additional endpoints include overall survival (OS), ORR, DOR, safety and quality of life. Independent, blinded reads of scans will be performed.

Eligibility criteria: Adults >18 yrs old, with metastatic breast cancer, triple-negative by most recent biopsy, measurable disease by CT or MRI as per RECIST1.1, ECOG performance score 0 or 1, adequate safety laboratories. Refractory/relapsed after ≥2 prior standard chemotherapy regimens for advanced disease, or >1 therapy for pts who progress within 12 months of adjuvant therapy. Pts must have received taxane and be eligible by investigator to receive at least one of the TPC agents. Pts with treated, non-progressive brain metastases are eligible.

Specific aims: To compare IMMU-132 to TPC as measured by PFS, OS, ORR, DOR,QOL, adverse events, safety laboratories, incidence of dose delays and reductions, and treatment discontinuations due to toxicity.

Statistical methods: Assuming a median PFS of 3 mo. and OS of 10 mo. with TPC vs. 5 and 15 mo. with IMMU-132, respectively, a study size of 328 patients has >95% and >80% power to detect a statistically significant difference in PFS and OS, respectively, between the two treatment arms.

Present accrual and target accrual: Trial enrollment will begin prior to SABCS 2017 with approximately 328 patients expected to be enrolled over 18 months at approximately 100 institutions in North America, Europe and potentially elsewhere.

Contact: Immu132@Immunomedics.com
Body: Background: Hormone receptor positive (HR+) disease is the most common subset of advanced breast cancer (BC). The majority of women with HR+ metastatic BC (MBC) develop resistance to endocrine therapy (ET), with a median survival of 2-3 years. A new strategy to treat HR+ MBC involves the combination of ET and a cyclin-dependent kinase 4/6 inhibitor (CDKi 4/6), which has demonstrated improved progression-free survival (PFS) in both first- and later-line MBC. Preclinical evidence in PI3K-mutant cell-line xenografts demonstrated that combinations of PI3K and CDK4/6i reduced intrinsic and adaptive resistance to ET, leading to tumor regression (Vara, 2004; Pfizer data). Inhibition of the PI3K/mTOR pathway by gedatolisib (G) may provide a new therapy to overcome ET resistance. These findings support developing the triplet combination of G with the CDKi 4/6 palbociclib (P)+letrozole (L) or fulvestrant (F) for the treatment of patients (pts) with ER+/HER2- BC.

Methods: This ongoing study in women with ER+/HER2- MBC, in first- and later-line settings, includes a dose-escalation (DE) to evaluate dose-limiting toxicities (DLTs, primary endpoint [pEP]) and determine the maximum tolerated dose and recommended phase 2 dose (RP2D) for a triplet regimen of G+P+L or G+P+F. The escalation rules follow the modified toxicity probability interval method (G doses: 180 and 215 mg IV weekly). Treatment assignment to the triplet is based on investigator decision and bone-only disease is permitted. After RP2D determination for each triplet, a 3-arm expansion for early signs of efficacy (ESOE) will investigate objective response rate (ORR) compared to historical controls [pEP] of Arm A) G+P+L in first-line, B) G+P+F in pts with no prior CDKi 4/6 in second-line and C) G+P+F in pts who have received prior CDKi 4/6. Pts receive G+P (125 mg oral daily for 21 days [D] on and 7 D off) + L (2.5 mg oral daily) or F (500 mg IM on D1, 15 of cycle [C] 1; D1 of C2 and then 500 mg IM on D1 of all 28-D cycles). Secondary endpoints include safety, tumor response (DE), PFS (ESOE), pharmacokinetics (PK), and biomarker correlations associated with the PI3K/mTOR pathway.

Results: 27 pts received G (180 mg/week) in combination with P+L (L cohort, n=12) or P+F (F cohort, n=15). Median prior therapies were: L cohort: 1 (range: 0-4); F cohort: 2 (range 1-5). The 3 most common, drug-related adverse events (%) were in L cohort: nausea (75), neutropenia (67), and stomatitis (67); F cohort: stomatitis (67), nausea (60), and neutropenia (53). C1 DLTs were: L cohort: grade (gr) 3 neutropenia (n=1); F cohort: gr 3 stomatitis (n=1). Preliminary rates of stable disease/partial response were: L cohort: 33%/16%; F cohort: 40%/13%. PK parameters and next-generation sequencing of PI3K-related mutations are pending.

Conclusions: G can be combined with P+L or P+F with manageable toxicity and promising preliminary antitumor activity, even in heavily pretreated pts. Dose escalation, followed by expansion for ESOE, is ongoing.

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Title: ATR inhibitor M6620 (formerly VX-970) with cisplatin in metastatic triple-negative breast cancer: Preliminary results from a phase 1 dose expansion cohort (NCT02157792)

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Body: Background: ATR is a critical regulator of the cellular response to replication stress; it signals DNA damage repair, mediated through homologous recombination. Many cancers depend on ATR to survive DNA damage. M6620 is a potent, selective inhibitor of ATR that augments the anticancer activity of cisplatin in preclinical triple-negative breast cancer (TNBC) models. Given the high prevalence of TP53 mutations in TNBC and limited platinum responsiveness in patients lacking a BRCA1/2 mutation, this study was designed to evaluate the safety and efficacy of M6620 in combination with cisplatin in an expansion cohort of patients with BRCA1/2 wild-type advanced/metastatic TNBC.

Methods: Eligible patients had advanced/metastatic ER-, PR-, and HER2- breast cancer with 0-2 prior non-platinum-based therapies and measurable disease per RECIST 1.1. First line patients were eligible if relapse occurred ≥3 months after prior (neo)adjuvant chemotherapy. Of a maximum 50 patients planned for enrollment, ≥30 were required to have BRCA1/2 germline wild-type status and basaloid molecular subtype tumors on central testing. Patients received intravenous cisplatin 75 mg/m² on day 1 with intravenous M6620 140 mg/m² on days 2 and 9 of each 21-day cycle. In patients intolerant of cisplatin or at investigator's discretion, cisplatin could be switched to carboplatin AUC 5 with M6620 90 mg/m².

Results: At the time of abstract submission, 35 female patients were enrolled in this study; 18 patients with confirmed BRCA1/2 wild-type and basaloid metastatic TNBC who received ≥1 cycle of study drug and had ≥1 baseline scan and ≥1 on-treatment scan at the time of the data cut were included in the primary efficacy analysis. Median progression-free survival (PFS) was 4.1 months (90% CI, 1.6-6.9 months). PFS was ≥6 months in 2 patients and ≥3 months in 8 patients. Preliminary unconfirmed objective response [complete response or partial response (PR)] was observed in 38.9% (90% CI, 19.9%-60.8%) of patients. All 7 patients with preliminary objective response had PR as best overall response; the longest duration of response was 183 days. Response was ongoing in 4 patients with PR at the time of data cutoff. Grade ≥3 related treatment-emergent adverse events occurred in 16 of 35 patients: neutropenia (n=8), anemia (n=5), vomiting (n=4), nausea (n=3), and, in 1 patient each, thrombocytopenia, neutrophil count decreased, platelet count decreased, hypokalemia, generalized weakness, rigors, and acute kidney injury.

Conclusions: Combination of M6620 and cisplatin shows encouraging antitumor activity and tolerability in patients with advanced/metastatic TNBC. The study is ongoing; updated safety and efficacy results will be presented.
2017 San Antonio Breast Cancer Symposium

Publication Number: OT2-07-08

Title: Substantially improving the cure rate of high-risk BRCA1-like breast cancer patients with personalized therapy (SUBITO) - an international randomized phase III trial

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Body: Background: An intact homologous repair (HR) pathway is essential for error-free repair of DNA double strand breaks (DSB). Preclinical and clinical data have shown that breast cancer (BC) cells with a defect in the HR pathway are a target for DNA DSB inducing agents and PARP inhibition. HR deficiency (HRD) causes genomic instability with a typical profile of DNA copy number aberrations (CNA) as seen in tumors of patients with a BRCA1 germline mutation. In the Netherlands Cancer Institute (NKI) the BRCA1-like test, a biomarker for HRD, was developed. The test calculates the probability that the genomic profile of DNA CNA of a breast tumor is similar to that of BRCA1 mutated BCs. When this probability is above a certain cutoff, depending on the platform, the test is positive in a small percentage of estrogen receptor positive and over 50% of triple negative BCs. In retrospective analyses, the test appeared to be a predictive biomarker for sensitivity to adjuvant intensified alkylating chemotherapy with autologous stem cell rescue (IA-CT). For example in stage III HER2-negative BRCA1-like BC, the 7-year recurrence free survival improved from 30% with 5 cycles of 5-fluorouracil-epirubicin-cyclophosphamide (FEC) to 78% when treated with 4 cycles of FEC followed by IA-CT (adjusted hazard rate 0.12; p=0.001). There was no benefit in non-BRCA1-like patients.

Prospective validation in a randomized phase III trial is eagerly awaited and will possibly result in a practice changing outcome. We hypothesize that, although treatment with IA-CT is controversial, by selecting the right patients, we may substantially improve survival in high risk BRCA1-like BC.

Eligibility criteria: Patients, 18 to 66 years old, with HER2-negative BRCA1-like or germline BRCA1 or BRCA 2 related stage III BC, who are fit to undergo IA-CT.

Trial Design: In this phase III trial, patients will be randomized to (neo)adjuvant treatment with 4 cycles of dose dense doxorubicin-cyclophosphamide (ddAC) followed by two cycles of IA-CT or followed by 4 cycles of carboplatin(q3)-paclitaxel(q1) and one year of olaparib (300mg twice daily). Adjuvant endocrine treatment is added according to guidelines. Primary outcome is overall survival (OS), analyzed with the intention to treat principle. To detect an improvement of 35% OS (40% vs 75%) with a 2-sided alpha of 0.05 and a power of 0.8 at 10 years, 174 patients should be enrolled. Secondary outcomes are toxicity, quality of life, cost-effectiveness and cognitive function. Additionally we plan to analyze potential biomarkers, like 53BP1 and cancer-immune interaction.

The SUBITO trial is part of a Coverage with Evidence Development program, which is a policy which allows conditional funding of a promising health intervention while more conclusive evidence is gathered to prove its (cost-)effectiveness. Since January 2017 we recruited 8 patients in 2 hospitals. Ten other hospitals, including two in France and one in Germany, are expected to start enrollment soon. The Medical Ethical Committee approved the trial. The trial is registered at clinicaltrials.gov (NCT02810743).

Contact information for people with a specific interest in the trial: e-mail: subito@nki.nl
Title: Detection and targeting of minimal residual disease in breast cancer to reduce recurrence: The PENN-SURMOUNT and CLEVER trials

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Body: Background:
Recurrent breast cancers arise from minimal residual disease (MRD): the pool of disseminated and circulating tumor cells (DTCs and CTCs) that survive in their host following treatment of primary breast cancer. Detection of DTCs in the bone marrow (BM) after treatment is strongly associated with an increased risk of recurrence. Through the analysis of novel genetically-engineered mouse models, we have generated a substantial body of evidence that autophagy and mTOR signaling play key roles in the survival of DTCs. Moreover, administration of agents that block these pathways in mice harboring MRD reduces DTC burden and concomitantly reduces tumor recurrence, providing the rationale for translating these findings to patients (pts).

Trial Design:
The PENN-SURMOUNT screening study uses a clinically validated IHC assay (DTC-IHC) to identify at-risk pts who harbor DTCs. DTC+ pts are eligible for enrollment on the CLEVER trial, which will determine the feasibility, safety and efficacy of administering hydroxychloroquine (HCQ) and/or everolimus (EVE) in DTC+ patients to target MRD and prevent recurrence. PENN-SURMOUNT is single center, prospective cohort study of pts who have completed therapy for primary breast cancer, are within 5 yrs of diagnosis and are at increased risk for relapse by virtue of nodal positivity, triple negative disease, ER+/Oncotype DX RS ≥ 25, or residual disease after neoadjuvant therapy. Pts undergo screening BM aspirate to test for DTCs following completion of adjuvant chemo and radiotherapy. The primary objective of the study is to determine the incidence and frequency of MRD in pts who have completed primary treatment for breast cancer and to ascertain eligibility for the CLEVER recurrence prevention trial.

CLEVER is a randomized, controlled, open label phase II pilot trial. Target enrollment is 60 pts, with 15 pts allocated to each of 4 treatment arms: HCQ (600 mg BID), EVE (10mg daily), combination HCQ/EVE, or control/observation. A cycle is 28 days of continuous dosing. After a 3-month observation period, control pts will be offered HCQ/EVE therapy for 6 cycles; thus, the control group is actually a delayed treatment group and all pts will receive treatment. Pts who demonstrate persistent DTCs after 6 cycles will continue on combination therapy for an additional 6 cycles. The primary endpoint is feasibility of administering HCQ, EVE or the combination in this population. Secondary objectives include safety, efficacy (DTC reduction), and 3-year RFS. The principal translational objective is to assess the utility of a novel DTC assay, "DTC-Flow", for more sensitive detection and response to study therapy, compared to DTC-IHC. Additional translational objectives include determining whether patient DTCs, CTCs, and cell-free circulating plasma tumor DNA (ptDNA) biologically reflect the primary tumor and predict response.

As of 5/23/17, 58 patients have been enrolled to PENN SURMOUNT, with a DTC-positivity rate of 22.6%; CLEVER opened in 2/2017; 11 patients are currently enrolled. Contact information: angela.demichele@uphs.upenn.edu

Key words: Recurrence, disseminated tumor cells, dormancy, minimal residual disease, autophagy, mTOR, Everolimus, hydroxychloroquine
Title: ATTAIN: Phase 3 study of etirinotecan pegol (EP) vs treatment of physician's choice (TPC) in patients (pts) with metastatic breast cancer (MBC) who have stable brain metastases (BM) previously treated with an anthracycline, a taxane, and capecitabine (ATC)

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Body: Background: EP is a next generation topoisomerase I inhibitor-polymer conjugate that provides continuous exposure to SN-38, the active metabolite. A BM mouse model showed high penetration and retention of SN-38 in CNS lesions, resulting in decreased size of CNS lesions and improved survival (OS) at concentrations achieved at the recommended dose in pts (Adkins BMC Cancer 2015). A Phase 3 trial (BEACON) of EP vs TPC in 852 pts with advanced BC did not meet its primary endpoint of OS (HR 0.087/uni037e p=0.08); a subset of 67 pts with stable BM showed improved OS (HR 0.51 [95% CI 0.30-0.86]/uni037e p<0.01) (Perez Lancet Oncol 2015). The current Phase 3 trial (ATTAIN) was designed for this subpopulation of pts having high unmet medical need.

Methods: Pts with MBC with locally treated stable BM will be randomized 1:1 to EP vs TPC in an open-label, randomized Phase 3 study. Eligibility includes ECOG PS 0 or 1; adequate organ function who received prior ATC (in neo/adjuvant or locally advanced/MBC setting) pts must have had ≥1 prior cytotoxic regimen for MBC (triple negative BC) ≥2 prior cytotoxic regimens and either 1 prior hormone therapy (HR+ BC) or 1 prior HER2 targeted therapy (HER2+ BC). Pts must have undergone definitive local therapy of BM (whole brain radiation [RT] stereotactic RT or surgical resection as single-agent or combination) signs/symptoms of BM must be stable with steroids unchanged or decreasing for ≥7 days prior to randomization. Primary endpoint is OS. Key secondary endpoints: ORR and PFS by RECIST v1.1 and RANO-BM, clinical benefit rate (ORR+SD ≥6 months) and QoL. Pts randomized to TPC will receive 1 of 7 IV cytotoxic agents. Pts are stratified by region, PS and receptor status. 350 pts will be randomized to obtain number of events required at 90% power to detect a statistically significant improvement in OS (hypothesizing HR=0.67) 1 interim analysis at 50% of deaths (130 events) will be performed. PK sampling and UGT1A1 testing will be performed in the EP arm; plasma ctDNA will be assessed for potential predictive markers of efficacy. Enrollment began early 2017. For enrollment information contact Dr. Alison Hannah, Dr. Mary Tagliaferri, or Minnie Kuo at StudyInquiry@nektar.com. NCT02915744
Title: Ixazomib in combination with carboplatin in pretreated women with advanced triple negative breast cancer, a phase I/II trial (AGMT MBC-10 trial)

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Body: Background: Triple-negative breast cancer (TNBC) comprises a heterogeneous group of diseases which are generally associated with a poor prognosis. Up to now, no targeted treatment beyond anti-VEGF therapy is approved for TNBC so far and cytotoxic agents are the mainstay for the treatment of advanced tumor stages. Ixazomib is a selective, and reversible inhibitor of the proteasome, which has been mainly investigated in the treatment of multiple myeloma. In a preclinical study triple-negative breast cancer cells were treated with bortezomib, a first generation proteaseome inhibitor, alone and in combination with cisplatin, which had a synergistic effect. Clinical data are available for carboplatin plus bortezomib in metastatic ovarian and lung cancers showing remarkable antitumor activity and good tolerability. Based on this rational, the MBC-10 trial will evaluate the toxicity profile and efficacy of ixazomib in combination with carboplatin in patients with advanced TNBC.

Trial Design: Patients with metastatic TNBC pretreated with at least one prior line of chemotherapy for advanced disease with a confirmed disease progression and measurable disease are eligible for this study. Patients will receive ixazomib in combination with carboplatin on days 1, 8, and 15 in a 28-day cycle. The phase I part of this study uses an alternate dose escalation accelerated titration design. After establishing the maximum tolerated dose (MTD), accrual continues to evaluate the efficacy and safety of the combination (phase II, including 41 evaluable patients). All patients will continue on study drugs until disease progression, unacceptable toxicity or discontinuation for any other reason. Primary endpoint of the phase II is overall response rate, secondary endpoints include safety profile, progression-free survival and quality of life. This trial is open for patient enrollment since November 2016 in six Austrian cancer centers. Accrual is planned to be completed within two years.

ClinicalTrials.gov Identifier: NCT02993094
Body: Introduction

Currently, risk management options for BRCA1 mutation carriers include risk-reducing mastectomies (RRM), and selective oestrogen receptor modulators (SERMs). However, the majority of BRCA1-associated tumours are oestrogen receptor negative (ER-ve). SERMs do not reduce the incidence of ER-ve tumours, therefore are unlikely to be effective in this group of women. Oestrogen and its metabolites have been shown to cause DNA damage, resulting in genomic instability in BRCA1 deficient breast cells, an early hallmark of BRCA1-related cancers. Based on this, we hypothesise that oestrogen suppression in BRCA1 carriers may reduce DNA damage in breast tissue, and thereby potentially reducing breast cancer risk.

Trial Design

The study is an interventional, non-randomised, crossover study without masking. The primary purpose is to assess the impact of these drugs on suppression of DNA damage and therefore their potential for use as chemopreventive agents in this setting. Pre-menopausal BRCA1 mutation carriers will be recruited from the Family History Clinic at Belfast City Hospital. The proportion of women who receive information packs who progress to trial entry will be recorded, with target recruitment of twelve women to the trial. Participants will complete quality of life questionnaires, provide blood and urine samples and undergo baseline ultrasound-guided breast biopsy. Half of these women will receive a SERM (tamoxifen) for 3 months, whilst the other half receives oestrogen suppression therapy (goserelin and anastrazole), and then provide repeat questionnaires and samples (blood, urine and breast biopsy). Treatment groups will cross over for a further 3 months treatment, with a final set of questionnaires and samples taken. Compliance with treatment and adverse events will be monitored throughout the study.

Eligibility Criteria

Inclusion criteria:
· Female
· Age ≥18 years
· Premenopausal
· Known pathogenic BRCA1 mutation
· Intact ovaries
· No previous breast/ovarian/other carcinoma
· No previous use of chemoprevention
· Willingness to use non-hormonal methods of contraception

Exclusion criteria:
· BRCA1 mutation of uncertain significance
· Contraindications to study drugs or breast biopsies
· Pregnancy or breastfeeding
· Inability to give informed consent
· Patient awaiting risk reducing surgery

Specific Aims

The primary objective is to assess the feasibility of treatments by measuring successful recruitment rates and compliance. The secondary objective is to establish tolerability of interventions through quality of life measurements and adverse event occurrence.

Exploratory objectives will assess the potential of treatments to reduce DNA damage through analysis of breast biopsies using comet assays and immunohistochemistry for 53BP1 and γH2AX (markers of DNA double-strand breaks). Oestrogen and metabolite levels will be measured in blood and urine samples using UPLC-MS/MS.

Statistical Methods

Not applicable due to nature and size of pilot study

Present accrual: 1 participant
Target accrual: 12 participants
Contact information for people with specific interest in trial: aideen.campbell@qub.ac.uk or s.mcintosh@qub.ac.uk
2017 San Antonio Breast Cancer Symposium

Publication Number: OT3-01-02

Title: Randomized controlled trial of web-based decision support tools for high-risk women and primary care providers to increase breast cancer chemoprevention

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Body: Background: Breast cancer chemoprevention with selective estrogen receptor modulators (SERMs) and aromatase inhibitors (AIs) is under-utilized despite several randomized controlled trials demonstrating a 40-65% decrease in breast cancer incidence among high-risk women. Reasons for low chemoprevention uptake include inadequate time for counseling, insufficient knowledge about SERMs and AIs, and concerns about side effects. Intervention trials of clinical decision support tools designed to increase chemoprevention uptake have been met with limited success. We have developed web-based decision aids (DAs), RealRisks for high-risk women and BNAV for primary care providers (PCPs). Our intervention differs from the prior literature in that we are targeting both patients and PCPs with personalized risk reports and education about the risks and benefits of chemoprevention. Our patient-centered decision aid is available in English and Spanish and has been rigorously tested in multi-ethnic women with varying health literacy. We hypothesize that standard educational materials combined with RealRisks and BNAV will increase uptake of SERMs or AIs among high-risk women in the primary care setting.

Trial Design: We are conducting a randomized controlled trial at Columbia University Medical Center (CUMC) in New York, NY, consisting of standard educational materials combined with RealRisks and BNAV or standard educational materials alone among 300 high-risk women stratified by Hispanic ethnicity and menopausal status. Women in the intervention arm are given access to the RealRisks DA, and, based on their responses, an action plan is generated summarizing their breast cancer risk profile, risks/benefits of SERMs and AIs, and personal preferences for chemoprevention. PCPs are given their patient's tailored risk report, which is the providers' view of the action plan, and are invited to access the BNAV tool.

Eligibility Criteria: 1) Women, aged 35-75 years; 2) 5-year invasive breast cancer risk ≥1.67% or lifetime risk ≥20% according to the Gail model (Breast Cancer Risk Assessment Tool) or history of lobular carcinoma in situ; 3) No prior use of SERM or AI; 4) No prior history of breast cancer; 5) PCP at CUMC; 6) English- or Spanish-speaking.

Specific Aims: The primary endpoint is chemoprevention uptake of a SERM or AI at 6 months based upon documentation in the electronic health record. Secondly, we use validated surveys to assess breast cancer and chemoprevention knowledge, accuracy of perceived breast cancer risk and worry, decision self-efficacy, and informed choice at baseline, 1 month, 6 months, and post-clinical encounter with the patients' PCP. PCPs will complete a 1-time survey on personal and professional characteristics and practice patterns.

Statistical Methods: With a total sample size of 300 (150 per arm), assuming a Type 1 error of 5% and a 10% drop-out rate (effective sample size of 270), we will have >80% power to detect a difference in chemoprevention uptake of 1% in the control arm and 10% in the active intervention arm.

Target Accrual: 300. Seventy-eight participants accrued as of June 2017. Accrual completion expected November 2018.

Contact: Katherine Crew, CUMC, kd59@cumc.columbia.edu
Development of cell-free nucleic acid-based tests for early detection of breast cancer: The STRIVE study

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Body: Background:
Mammography (digital 2D or digital 3D/tomosynthesis) is the cornerstone of current screening strategies for breast cancer, but new approaches are needed to further reduce the proportion of cancers diagnosed at advanced stages and more effectively identify those women in need of additional testing and biopsies. Circulating cell-free nucleic acids (cfNAs) shed from tumors, isolated from peripheral blood, and analyzed with ultra-deep and broad sequencing of cancer-associated genes, have great potential for early cancer detection. The ultimate goal is to develop blood cfNA cancer screening tests for use in conjunction with established risk factors and/or radiographic features for improved cancer detection. Development of these tests requires large, well-annotated cohorts of asymptomatic participants with adequate volumes of prediagnostic blood. The STRIVE Study cohort was recently established to train and validate cfNA-based tests for early detection of breast cancer.

Eligibility criteria and trial design: The STRIVE Study is a new prospective, multi-ethnic mammography cohort that will recruit 120,000 subjects from 15+ US breast cancer screening centers (including Mayo Clinic and Sutter Health sites). Eligibility criteria require only that a participant has a scheduled routine screening mammogram at a participating center and has not received a biopsy prior to the research blood draw. Participants are recruited within 28 days of screening mammography (digital or tomosynthesis), consent electronically, provide blood samples, and complete an on-line risk factor questionnaire. Participants will be followed for all cancer diagnoses, cancer recurrences, and death for at least 5 years. Pertinent medical record information, imaging findings (including breast density), and follow-up data will be transferred electronically to a central database throughout the study period. Additional blood samples will be collected from participants with abnormal mammogram results, or who are diagnosed with cancer, to document and better understand the evolution of cfNA signals. Recruitment began in February 2017.

Primary Aims: To train and validate a cfNA blood-based test to identify breast cancer overall in a cohort of women undergoing screening mammography.

Statistical Methods: The study will be divided into a training phase (1/3 of participants) and an independent clinical validation phase (remaining 2/3 of participants). In the training phase, statistical machine learning techniques will be used to develop algorithms incorporating cfNA signals, clinical characteristics, or radiological features. In the validation phase, the prespecified locked algorithm developed from the training phase will be clinically validated in an independent group of women.

Contact information for people with a specific interest in the trial: Additional details regarding the STRIVE Study are available on the ClinicalTrials.gov website (NCT03085888). For site-specific questions, please call 844-366-9738 for the Mayo Clinic and 1-855-578-7483 for Sutter Health.
**Title:** Preference-Tolerant randomized trial of risk-based vs. annual breast cancer screening: WISDOM study in progress

**Body:** Purpose: Women Informed to Screen Depending on Measures of risk (WISDOM) trial is a pragmatic study comparing two real world approaches to clinical care for breast screening: annual screening versus personalized screening. The novelty of the personalized arm of the study is that we are combining known risk factors (age, family history, history of breast disease, ethnicity, BIRADS breast density, and genetics) into a single risk assessment model. All components of the model have been tested and established, but have never been used jointly. The goal of the WISDOM study is to examine the effectiveness of personalized breast cancer screening and to bring objective recommendations to the current mammography screening debate.

Methods: The WISDOM trial will enroll 100,000 women with a preference-tolerant design that will determine if risk-based screening vs. annual screening, is as safe, less morbid, enables prevention, and is preferred by women. Women 40 - 74 years of age with no history of breast cancer or DCIS, and no previous double mastectomy can join the study from the WISDOM Study website (wisdomstudy.org). All participants sign up, elect randomization or self-select the study arm, provide electronic consent using DocuSign (eConsent), and sign a Medical Release Form. For all participants, 5-year risk of developing breast cancer is calculated according to the Breast Cancer Screening Consortium (BCSC) model. For participants in the personalized arm, the overall 5-year risk BCSC score is combined with a Polygenic Risk Score, based on a genetic test including mutations in 9 genes (BRCA1, BRCA2, TP53, PTEN, STK11, CDH1, ATM, PALB2, and CHEK2) and a panel of 75 common single nucleotide polymorphisms known to increase breast cancer risk. Risk stratification will determine frequency of screening. The study is registered on ClinicalTrials.gov as NCT02620852.

Results: As of June 12th 2017, the WISDOM study is live at all UC medical centers and recruitment is open to all eligible women in California. Up to date 4,769 eligible women registered at all sites. 2,823 women have consented in the trial. 64% were randomized and 36% chose their screening arm. A pilot was conducted to test the logistics of online participation and examine the acceptance of the study design and approach. We are partnering with health insurance companies and self-insured companies to reach our recruitment goal.

Conclusions: Enrollment will be completed by end of 2018.

Acknowledgment: support by the Patient-Centered Outcomes Research Institute (PCORI), PCS-1402-10749 to L.J.E.

(*) Authors equally contributed to this work.
A phase I of olaparib with radiation therapy in patients with inflammatory, loco-regionally advanced or metastatic TNBC (triple negative breast cancer) or patient with operated TNBC with residual disease

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Body: Background and discussion: TNBC shares clinical and pathological features with hereditary BRCA1-related breast cancers, and in sporadic TNBC; dysregulation of BRCA1 has been frequently observed together with other defects in homologous recombination pathways. Preclinical studies have shown that breast cancer cell lines with a triple-negative phenotype are more sensitive to PARP1 inhibitors compared with non-TNBC cells. These lines of evidence provide a strong rationale for developing a new therapeutic approach to TNBC based on targeting the DNA-repair defects via PARP inhibition in these cancers that the most aggressive are the inflammatory, loco-regional advanced and metastatic breast cancer, as well as operated patients with residual disease (after primary systemic treatment-PST).

The aim of this study is to determine the Maximal Tolerated Dose of Olaparib administered with concurrent loco regional RT in the previously described population of patients.

Trial design: Olaparib (oral administration) will be administered at a starting dose of 50 mg bid. The other dose levels will be: 100 mg bid, 150 mg bid, 200 mg bid. The 25 mg bid dose will be included in the model to deal with unexpected high toxicity of the starting dose. Seven days prior to their first fraction of radiation therapy, patients will begin taking Olaparib at the assigned dose twice daily each day. All patients will receive radiotherapy on day 8 after the start of Olaparib of 50 Gy to the whole breast (or chest wall) with or without lymph nodes (LN) in 25 daily fractions and 5 weeks.

Eligibility Criteria: Women aged >18 years with histologically confirmed TNBC with loco-regional RT indication as:

- Non-operated: Inflammatory and/or advanced BC (T≥3 and/or N≥1) BC in progression during PST (containing anthracyclines or taxanes or the combination of both or containing platinum-based chemotherapy) or inoperable after PST.
- Non operable metastatic BC (all T, all N, M1; with evaluable disease).
- Or patients operated after PST and surgery with residual disease (non-pCR and pN+ disease, evaluable according to RECIST 1.1 criteria).

Specific aims

To assess the safety profile of Olaparib administered with concurrent RT. This study should be completed by a methylation study of BRCA1 and RAD51 promoters.

Statistics Phase I dose-finding based on toxicity will be conducted in a sequential and adaptive Bayesian scheme, using the method of Time-to-event Continual Reassessment Method to determine the Maximum Tolerated Dose (MTD) of Olaparib associated with RT. The primary endpoint is Dose-Limiting Toxicity (DLT) occurring within 6 weeks after the end of RT (12 -13 weeks from the first drug intake, depending on the period of the radiotherapy treatment). Dose allocation will be centrally defined, based on DLT observed in all patients previously evaluated, by modeling the probability of DLT. An empiric model will be used for the dose-toxicity relationship. No intra-patient dose-escalation is permitted. No dose skipping in escalation is permitted. The MTD is defined as the dose associated with 25% of DLT.

Target accrual: Twenty-four to 30 pts are expected to be enrolled.

Contact: youlia.kirova@curie.fr
The DORA trial: A non-comparator randomised phase II multi-center maintenance study of olaparib alone or olaparib in combination with durvalumab in platinum treated advanced triple negative breast cancer (TNBC)

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Body: Background: Recent data from the OlympiaD study revealed an improvement in response rate and progression-free survival (PFS) with the PARP inhibitor (PARPi) olaparib vs. standard of care chemotherapy in patients with metastatic breast cancer who harbor germline BRCA (gBRCA) mutations. Maintenance PARPi has improved median PFS in relapsed ovarian cancer regardless of gBRCA mutation status or HRD status. The similarities between the molecular aberration profiles of high-grade serous ovarian cancer and TNBC invites exploration of maintenance PARPi in mTNBC. Furthermore, because durable responses have been reported in subsets of patients with metastatic TNBC (mTNBC) with checkpoint blockade and because high mutational load is associated with both gBRCA and TNBC, these patients may be particularly susceptible to immunotherapy with PARPi. Thus, we hypothesize that olaparib either alone or in combination with the PD-L1 inhibitor durvalumab will be active in TNBC subjects who have responded to platinum-based chemotherapy.

Trial design: DORA is a non-comparator randomized, international, multicenter phase II study designed to explore the efficacy of olaparib with or without durvalumab as maintenance therapy in platinum-treated mTNBC. Subjects will be enrolled following four cycles of treatment with a platinum-based (cisplatin or carboplatin) chemotherapy as single agent or combination therapy. Subjects deriving clinical benefit (CR / PR / SD) with platinum-based therapy as determined by the treating physician will be eligible and randomized in a 1:1 ratio. Patients in arm 1 will receive olaparib orally 300mg BID continuously and in arm 2 will receive olaparib orally 300mg BID continuously in combination with durvalumab 1500mg IV every 4 wks. Tumor responses will be assessed every 8 wks.

Eligibility criteria: Subjects with mTNBC who are receiving platinum-based chemotherapy and who have had no more than 2 lines of chemotherapy in the metastatic or advanced setting, with one of those being a platinum, will be included in this trial. Eligible patients must have been assessed by their treating physicians to have derived clinical benefit with platinum based therapy. Archival tissue or fresh biopsy samples are mandated for biomarker analyses.

Aims: The primary endpoint is PFS; the key secondary endpoint is overall survival.

Statistical methods: The sample size is calculated based upon data derived from contemporary trials of chemotherapy in mTNBC. In both arms of the study, it is proposed to test a null hypothesis of a median PFS of 2 months against an alternative hypothesis of a median PFS of 4 months; there is no intention to make a formal statistical comparison between the two treatment arms. To test this hypothesis, assuming an exponential PFS distribution, use of an exponential MLE test, a two-sided significance level of 5% and a power of 90%, 25 subjects are required per arm.

Target accrual: To allow for a drop-out rate of approximately 20%, the sample size per arm will be inflated to 30 subjects. We plan to enroll approximately 60 subjects with mTNBC from 6 centers.

ClinicalTrials.gov Identifier: NCT03167619
**Title:** PARTNER randomised, phase II/III trial to evaluate the safety and efficacy of the addition of olaparib to platinum based neoadjuvant chemotherapy in triple negative and/or germline BRCA mutated breast cancer patients

Jean Abraham¹, Anne-Laure Vallier², Wendi Qian², Louise Grybowicz², Stanly Thomas², Andrea Machin², Caron Harvey², Edmund Chiu², Karen McAdam², Luke Hughes-Davies², Rebecca Roylance³, Ellen Copson⁴, Anne Armstrong⁵, Elena Provenzano², Marc Tischkowitz¹, Emma McMurtry⁶ and Helena Earl¹. ¹University of Cambridge, Cambridge, Cambridgeshire, United Kingdom; ²Cambridge University Hospitals NHS Foundation Trust, Cambridge, Cambridgeshire, United Kingdom; ³University College London, London, United Kingdom; ⁴University of Southampton, Southampton, Hampshire, United Kingdom; ⁵The Christie NHS Foundation Trust, Manchester, United Kingdom and ⁶AstraZeneca, Macclesfield, Cheshire, United Kingdom.

**Body:**

**Background:** No specific targeted therapies are available for Triple Negative Breast Cancers (TNBC), an aggressive and diverse subgroup. The basal TNBC subgroup show some phenotypic and molecular similarities with germline BRCA (gBRCA). In gBRCA patients, and potentially other homologous recombination deficiencies, these already compromised pathways may allow drugs called PARP inhibitors (olaparib) to work more effectively.

**Aims:** To establish if the addition of olaparib to neoadjuvant platinum based chemotherapy for basal TNBC and/or gBRCA breast cancer is safe and improves efficacy (pathological complete response (pCR)).

**Trial design:** 3 stage open label randomised phase II/III trial of neoadjuvant paclitaxel and carboplatin +/-olaparib, followed by clinicians' choice of anthracycline regimen.

**Stage 1 and 2:** Patients are randomised (1:1:1) to either control (3 weekly carboplatin AUC5/weekly paclitaxel 80mg/m² for 4 cycles) or one of two research arms with the same chemotherapy regimen but with two different schedules of olaparib 150mg BD for 12 days.

**Stage 3:** Patients are randomised (1:1) to either control arm or to the research arm selected in stage 2.

**Methods:**

**Stage 1 Safety:** both research arms combined.

**Stage 2 Schedule selection criteria:** pCR rate and completion rate of olaparib protocol treatment. It is a “pickthewinner” design with 53 patients in each research arm. This allows a 90% power, 5% onesided significance level to test null hypothesis of pCR ≤35% versus an alternative hypothesis of pCR ≥55% in each of the research arms.

**Stage 3 Efficacy:** anticipated pCR ~55-60% for all trial patients and ~60-65% for gBRCA patients. The trial is powered to detect an absolute improvement of 15% (all patients) and 20% (gBRCA patients) by adding olaparib to chemotherapy (enriched design). TNBC patient recruitment will be capped, to ensure required gBRCA patients are enrolled. Enrichment design is applied with overall significance level 0.05(α) = 0.025(αall) + 0.025(αgBRCA) and 80% power.

**Target accrual:** 527 [gBRCA 220] Current accrual: 56 Sites activated: 15 [expected number of sites 30-50].
Title: LUCY: A phase IIIb, single-arm, open-label multicenter study of olaparib in patients with HER2-negative metastatic breast cancer and a germline BRCA1/2 mutation

Karen Gelmon¹, Graham P Walker² and Graham V Fisher². ¹British Columbia Cancer Agency, Vancouver, Canada and ²AstraZeneca, Cambridge, United Kingdom.

Body: Background
Olaparib (Lynparza) is a PARP inhibitor with activity in patients with advanced cancers who have a germline BRCA1 and/or BRCA2 (gBRCA) mutation and is licensed for use in gBRCA-mutated recurrent ovarian cancer. The Phase III OlympiAD trial (NCT02000622) in HER2-negative metastatic breast cancer (mBC) patients with a gBRCA mutation showed a significant progression-free survival (PFS) improvement in favor of olaparib compared with physician's choice of chemotherapy treatment (hazard ratio [HR] 0.58; 95% confidence interval [CI] 0.43–0.80; P<0.001; 7.0 vs 4.2 months, respectively) (Robson et al. NEJM 2017). The LUCY trial (EudraCT number: 2017-001054-34) has been initiated to further evaluate the clinical effectiveness of olaparib in a real-world setting, and to help inform and guide clinical practice.

Trial design
LUCY is an open-label, single-arm, multicenter, international Phase IIIb trial. All patients will be treated with open-label olaparib tablets (300 mg twice daily) until disease progression, unacceptable toxicity, or other discontinuation criteria.

Eligibility criteria
Eligible patients aged ≥18 years will have a gBRCA mutation and HER2-negative mBC. Patients are required to have received a prior taxane or anthracycline in either the adjuvant or metastatic setting, but should not have received >1 line of chemotherapy in the metastatic setting. Hormone receptor-positive patients are also required to have received and progressed with ≥1 prior endocrine therapy. Patients will be required to have an expected survival of >6 months.

Objectives
The primary objective is to evaluate the clinical effectiveness of olaparib through investigator-defined assessment of PFS (radiological, symptomatic, or clear progression of non-measurable disease). Secondary objectives will include assessments of overall survival (OS), time to first/second subsequent therapy, time to second progression and time to study treatment discontinuation, as well as assessment of clinical response rate and duration of clinical response. Safety and tolerability will also be described.

Statistical methods
Approximately 2500 patients will be screened to identify 250 patients with a gBRCA mutation. The primary analysis of PFS will be performed after 160 progression events: assuming a median PFS of 7 months, the predicted 95% CI for the median is 6.0–8.2 months. Analysis of OS and updated PFS will be performed after 160 deaths: assuming a median OS of 19 months, the predicted 95% CI for the median is 16.3–22.2 months. PFS and OS will be summarized using a Kaplan–Meier plot, from which the median and 95% CI data will be calculated.

Present accrual
Screening is expected to take place across ~180 sites in 17 countries.

Summary
LUCY will provide further data on the efficacy of olaparib in the real-world setting of mBC in patients with gBRCA mutation.
2017 San Antonio Breast Cancer Symposium

Publication Number: OT3-05-01

Title: TBCRC 039: Phase II study of combination ruxolitinib (INCB018424) with preoperative chemotherapy for triple negative inflammatory breast cancer

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Body: Background: Stage III triple negative (TN) inflammatory breast cancer (IBC) is associated with a poor prognosis evidenced by a 15 month (mo) median disease free survival (DFS) and overall survival (OS) of 34 mo. The substantial incidence of developing distant metastasis may be due to the prevalence of cancer cells with stem cell-like features (e.g. CD44+/CD24-) in TNIBC. The transcriptional pathway JAK2/STAT3 is associated with the survival of CD44+/CD24- cells, and preclinical data demonstrates overexpression of activated STAT3 (pSTAT3) in > 95% of TNIBC. Preclinical studies have shown that ruxolitinib (Incyte® Corporation), an approved JAK1/JAK2 inhibitor, suppresses pSTAT3 in IBC patient derived xenograft models, and when combined with paclitaxel, results in a synergistic reduction in tumor weight. Given the lack of a known therapeutic target in TNIBC, this preoperative proof of principle study exploits the survival mechanism of CD44+/CD24- stem cells prevalent in this disease, by combining ruxolitinib (Rux) with paclitaxel (T) followed by doxorubicin/cyclophosphamide (AC).

Methods: Up to 64 pts with newly diagnosed stage III TNIBC (cT4d, any N, M0) are eligible if they have adequate organ function and are willing to undergo 2 research biopsies (rbx) of the affected breast. Following baseline rbx, pts are randomized to a 7 day (d) run-in phase of Rux vs Rux (15 mg bid) + T (80 mg/m²/wk x 1). A 2nd rbx is obtained after the run-in phase. Pts randomized to RuxT continue to receive a total of Tx12 wks + Rux. Pts randomized to Rux alone, are re-randomized to receive Tx12 wks + Rux vs Tx12 wks alone. Following T, all pts receive AC (A-60 mg/m², C-600 mg/m²) every 14 d x 4. Pts proceed to modified radical mastectomy (MRM) followed by chest wall/regional lymph nodes radiation therapy.

Correlatives: To assess the effect of JAK inhibition with Rux on pSTAT3 and STAT3 related gene expression, molecular and genomic markers (e.g. RNA-seq, ChiPseq, FISH) will be determined in each rbx and residual tumor at MRM. The relative frequency and topology of CD44+/CD24- cell population and pSTAT3 expression by IHC will also be assessed in these tumor specimens. IL-6 and CRP plasma concentrations will be measured at baseline, prior to T and AC and prior to MRM.

Statistics: The primary endpoint is change in markers of JAK/STAT inhibition. If the proportion of rbx exhibit a biologic response to Rux alone (i.e. change from pSTAT3 expression to pSTAT3 negative) is ≤10%, then Rux alone is minimally effective on JAK inhibition vs alternative hypothesis that Rux inhibits JAK if the proportion of biologic response is ≥33%. If ≥5/25 rbx treated with Rux alone have a biologic response then the hypothesis that biologic response is ≤10% is rejected with an error rate of 0.098 (target 0.10). If ≤4/25 rbx have a biologic response then the hypothesis that biologic response is ≥33% is rejected with an error rate of 0.05 (target 0.10). Biologic response of rbx with Rux alone will also be compared with the proportion of biologic response to RuxT (33% vs. 66% based upon presumed synergy with RuxT). Secondary endpoints are clinical: pathologic complete response in breast/lymph nodes, Residual Cancer Burden, DFS and OS. Clinical Trial Information: NCT02876302.
BYLieve: A phase 2 study of alpelisib with fulvestrant or letrozole for treatment of PIK3CA mutant, hormone receptor-positive (HR+), human epidermal growth factor receptor 2-negative (HER2−) advanced breast cancer (aBC) progressing on/after cyclin-dependent kinase (CDK)4/6 inhibitor therapy

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Body: Background: Endocrine therapy (ET) is the standard of care for treatment of HR+, HER2− aBC. However, ET resistance occurs frequently leading to disease progression. Dysregulation of the PI3K/AKT/mTOR pathway, specifically mutations in PIK3CA, the gene encoding the p110α subunit of PI3K, has been implicated in ET resistance. In a phase 1 study, alpelisib, a PI3Kα-specific inhibitor, in combination with fulvestrant has shown antitumor activity in patients with PIK3CA mutant, HR+, HER2− aBC. The present BYLieve (NCT03056755) study aims to assess the efficacy and safety of alpelisib + fulvestrant/letrozole in PIK3CA-mutant, HR+, HER2− aBC progressing on/after prior CDK4/6 inhibitor (CDK4/6i) therapy.

Methods: BYLieve is a phase 2, multicenter, open-label, 2-cohort, non-comparative study. Men and postmenopausal women (≥ 18 years) with PIK3CA-mutant, HR+, HER2− locally advanced or metastatic breast cancer that has progressed on/after prior CDK4/6i therapy are eligible. Other eligibility criteria include ≥ 1 measurable lesion (RECIST v1.1) or predominantly lytic bone lesion; ECOG PS ≤ 2; and no prior PI3K inhibitor therapy. Patients are allocated to 2 cohorts based on the prior ET partner (aromatase inhibitor (AI) or fulvestrant) used in combination with CDK4/6i. Cohort A (patients who had received CDK4/6i + AI): oral alpelisib (300 mg once daily) + intramuscular fulvestrant (500 mg on days 1 and 15 of cycle 1, and day 1 of cycles ≥ 2 [28-day cycles]) and cohort B (patients who had received CDK4/6i + fulvestrant): oral alpelisib (300 mg once daily) + oral letrozole (2.5 mg once daily). Study treatment will continue until disease progression or intolerable toxicity. The primary end point is the proportion of patients who are alive without disease progression at 6 months (RECIST v1.1; local assessment), which will be evaluated separately in each cohort and presented together with 2-sided 90% confidence intervals using Clopper and Pearson (1934) exact method. Evidence of treatment effect will be demonstrated if the lower bound of the 90% CI is greater than 30%. A total sample size of 80 patients in each cohort is planned. Secondary end points include progression-free survival (PFS), PFS on next-line treatment (PFS2), overall response rate, clinical benefit rate, duration of response, safety, and tolerability. Detection of frequency of PIK3CA mutations in ctDNA and its correlation with response is an exploratory end point.
Title: POLARIS: Palbociclib (P) in hormone receptor-positive (HR+) advanced breast cancer: A prospective multicenter noninterventional study

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Body: Background: P is a novel cyclin-dependent kinase 4/6 inhibitor approved in the United States and Canada in combination with endocrine therapy for HR+/human epidermal growth factor receptor 2–negative (HER2-) advanced breast cancer (ABC). Despite promising trial results, not all patients respond to P. Moreover, despite a median age at diagnosis of 62 years, elderly patients are underrepresented in targeted therapy trials, including the PALOMA studies assessing P. It is important to understand P use in real-world practice settings, including tolerability and outcomes in the vulnerable older population. In addition, understanding the mechanisms of P response or resistance is critical to identify clinical factors and biomarkers that can predict which patients will benefit from P. This multicenter observational and biomarker study will seek to address these and other data gaps.

Trial Design: This is a prospective, noninterventional study of 1500 patients treated with P from 100 US and 10 Canadian sites. Study duration will span 2 years of recruitment and 3 years of follow-up after P treatment, until patient withdrawal from the study or death. Study participation is not intended to alter routine treatment; all treatment decisions, including type and timing of disease monitoring, are at the discretion of the treating physician and patient.

Eligibility: Eligible patients are aged ≥18 years with a diagnosis of adenocarcinoma of the breast with (1) evidence of advanced or metastatic disease not amenable to treatment with curative intent, (2) documented HR+/HER2- status, and (3) planned treatment with P. Patients with a life expectancy <3 months at initial diagnosis, those participating in interventional trials, and those receiving active treatment for malignancies other than ABC at enrollment are ineligible.

Aims: In a large real-world cohort of HR+/HER2- ABC patients treated with P in routine clinical practice, this study aims to assess the following: prescribing and treatment patterns for ABC before, during, and after P therapy; overall clinical response to P; biomarker assessment investigating potential mechanisms of response and resistance to P based on genomic analyses of blood samples; patient quality of life, as measured by the European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire Core 30; geriatric assessments in patients aged ≥70 years at enrollment based on the G8 Geriatric Screening Tool and the Activities of Daily Living questionnaire; and sequencing of treatment for metastatic disease. Other outcomes to be assessed include survival and toxicity.

Methods: Data will be collected from routine clinical assessments. Patients will have the option to provide blood samples drawn at standard-of-care intervals at baseline, during P treatment, and at the end of treatment for potential biomarker identification. Analyses will be primarily descriptive, with point estimates and confidence intervals as well as Kaplan-Meier methods used to assess time-to-event outcomes.

Accrual: Presently, 46 patients from 20 sites are enrolled.

Funding: Pfizer Inc.
Title: A randomized, open-label, multi-center phase IV study evaluating palbociclib plus endocrine treatment versus a chemotherapy-based treatment strategy in patients with hormone receptor-positive, HER2-negative metastatic breast cancer in a real world setting (PADMA)

Sibylle Loibl¹, Jana Barinoff², Sabine Seiler¹, Thomas Decker³, Carsten Denkert², Anne-Claire Hardy-Bessard⁴, Elżbieta Senkus-Konefka⁵, Francesco Cognetti⁶, Carlo Palmieri⁷, Karen Gelmon⁸, Kristina Luebbe¹, Jenny Furlanetto¹, Volkmar Mueller¹⁰, Christoph Mundhenke¹¹, Marcus Schmidt¹², Gunter von Minckwitz¹, Mathias Uhlig¹, Nicole Burchardi¹ and Marc Thill¹³.

Body: Background:
Although endocrine therapy (ET) is recommended as first-line therapy for hormone receptor (HR)-positive, HER2-negative metastatic breast cancer (MBC) up to 50% of patients receive chemotherapy in this setting. Meanwhile new targeted treatment options for combination with ET have been developed and endocrine-based therapy with the CDK4/6 inhibitor Palbociclib (P) improves the progression free survival (PFS) of ET alone by about 50%. So far, there is no data comparing chemotherapy with or without maintenance ET and ET in combination with P as first-line therapy. Patients included in clinical trials are often criticized not to mirror the general breast cancer population and every-day clinical practice due to rigid inclusion and exclusion criteria, limited number of treatment options, strict monitoring intervals and study assessments.

Methods:
PADMA trial is a so called low intervention trial with no rigid inclusion and exclusion criteria, and study assessments. Patients with first-line HR+/HER2- MBC who are candidate for mono-chemotherapy will be eligible to receive either P plus ET per label or mono-chemotherapy per investigator’s choice with or without maintenance ET (1:1 randomization). Primary objective is to compare the time-to-treatment failure (TTF) for patients randomized to receive the mono-chemotherapy treatment strategy versus those randomized to receive P and ET. TTF is defined as time from randomization to discontinuation of treatment due to disease progression, treatment toxicity, patient’s preference, or death. Main secondary objectives are progression free survival, overall survival at 36 months, amongst other time to event endpoints as well as toxicity and compliance. All patients receive a specific mobile device (PADMA-Phone) and a validated wearable device (ActiWatch) in order to collect data regarding sleep and activity levels, patient well-being and health care utilization (number and duration of phone calls, and patient visits to investigator site) for assessment of daily monitoring treatment impact (DMTI).

Results:
Overall, 360 patients will be accrued to show an improved TTF for P in combination with ET compared to mono-chemotherapy of investigator’s choice with or without maintenance ET. Recruitment will start in QIII/2017 and is planned for approximately 18 months in 100 sites in Germany, Spain, Poland, Italy, France, UK and Canada.

Conclusions:
The aim of PADMA is to demonstrate that an endocrine-based strategy consisting of ET plus P is superior to a chemotherapy-based strategy as first-line therapy in women with HR+/HER2- MBC in a real world setting. Assessment of patient-reported outcome, health care utilization, and sleep and activity levels will deliver important information on the differences between endocrine-based and chemotherapy-based treatment.
**2017 San Antonio Breast Cancer Symposium**

**Publication Number:** OT3-05-05

**Title:** MonarchE: A randomized, open-label, phase 3 study of abemaciclib combined with standard adjuvant endocrine therapy versus standard adjuvant endocrine therapy alone in patients with high risk, node positive, early stage, HR+, HER2- breast cancer

Priya Rastogi¹, Masakazu Toi², Nadia Harbeck³, Nawel Bourayou⁴, Martin Frenzel⁵ and Stephen Johnston⁶. ¹University of Pittsburgh Medical Center, Pittsburgh, PA; ²Graduate School of Medicine, Kyoto University, Kyoto, Japan; ³Breast Center, University of Munich (LMU), Munich, Germany; ⁴Eli Lilly and Company, Paris, France; ⁵Eli Lilly and Company, Indianapolis, IN and ⁶The Royal Marsden NHS Foundation Trust, London, United Kingdom.

**Body:** Background: Abemaciclib, an oral, selective inhibitor of cyclin-dependent kinases 4 and 6 dosed on a twice daily continuous schedule, has demonstrated clinical efficacy and tolerability in patients (pts) with hormone receptor-positive (HR+), human epidermal growth factor receptor 2-negative (HER2-) advanced breast cancer when administered as monotherapy (MONARCH 1) and in combination with endocrine therapy (ET) in MONARCH 2 and MONARCH 3. In neoMONARCH, abemaciclib plus anastrozole as neoadjuvant therapy reduced the breast tumor cell proliferation marker Ki67 to a greater extent than anastrozole alone after 2 weeks of treatment. Endocrine monotherapy is the current standard of care in the adjuvant setting. However, a proportion of pts relapse despite this therapy. A population with a higher risk of recurrence (15% at 5 years) may be identified based on the clinical and pathological characteristics of disease. Optimizing adjuvant therapy for these pts is an important need.

**Trial Design:** MonarchE (NCT03155997) is a multicenter, randomized, open-label Phase 3 trial that will evaluate the potential for abemaciclib to enhance adjuvant ET. Pts will be randomized 1:1 to abemaciclib 150 mg twice daily continuous schedule plus standard of care (SOC) adjuvant ET versus SOC adjuvant ET alone and stratified by prior chemotherapy (neoadjuvant, adjuvant, or none), menopausal status (pre- or post-), and region (N. America/Europe, Asia, or other). Pts may have started ET within 8 weeks prior to randomization. Pts will receive abemaciclib for up to 2 years in combination with ET per physician's choice (such as tamoxifen or an aromatase inhibitor, +/- ovarian suppression). ET alone will be continued as clinically indicated. All randomized pts will be followed for a total of 10 years.

**Eligibility Criteria:** Eligible pts (male or female) must have early stage resected HR+, HER2- invasive breast cancer with either ≥ 4 positive pathological axillary lymph nodes (pALNs), or 1 to 3 positive pALNs and at least one of the following high risk markers: primary tumor size ≥5 cm, histological grade 3 tumor, or centrally assessed Ki67 index of ≥20% (in a subset of pts). Pts must have completed definitive locoregional therapy (+/- (neo)adjuvant chemotherapy) and be randomized no more than 12 weeks after completion of last non-ET (surgery, chemotheraphy, or radiotherapy). Pts must have tumor tissue available for biomarker analysis prior to randomization.

**Specific Aims:** The primary objective of monarchE is to evaluate invasive disease-free survival (IDFS) per the STEEP System.¹ Secondary objectives include evaluation of IDFS in pts with Ki67 index of ≥20%, distant relapse-free survival, overall survival, safety, pharmacokinetics, and pt health outcomes.

**Statistical Methods:** Assuming an IDFS hazard ratio of .73, the study is powered to approximately 80% to test the superiority of abemaciclib plus standard ET at a 1-sided α=0.025 using a stratified log-rank test.

**Target accrual:** Approximately 3580 pts

**Contact information:** 1-877-285-4559

**Reference:**
Title: EarLEE-2: A phase 3 study of ribociclib + endocrine therapy (ET) for adjuvant treatment of patients with hormone receptor-positive (HR+), human epidermal growth factor receptor 2-negative (HER2–), intermediate-risk, early breast cancer (EBC)

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Body: Background: Adjuvant ET with or without chemotherapy reduces the risk for recurrence of HR+, HER2– EBC. However, recurrence is still common, especially in patients with adverse clinical and pathologic features. In the phase 3 MONALEESA-2 trial, the cyclin-dependent kinase 4/6 inhibitor ribociclib (LEE011), in combination with letrozole, prolonged progression-free survival versus letrozole plus placebo in postmenopausal women with HR+, HER2– advanced breast cancer and no prior therapy for advanced disease (HR = 0.56, 95% CI, 0.43-0.72; \( P = 3.29 \times 10^{-6} \); Hortobagyi et al. N Engl J Med. 2016). EarLEE-2 is investigating the efficacy and safety of ribociclib with ET versus placebo with ET as adjuvant treatment in patients with intermediate-risk EBC.

Trial design: In this double-blind, placebo-controlled, phase 3 adjuvant trial, ~4,000 women and men with fully resected, intermediate-risk, HR+, HER2– EBC (defined as AJCC 8th ed. Prognostic Stage Group II) are being randomized 1:1 to oral ribociclib (600 mg/day, 3 weeks on/1 week off for ~24 months) plus ET or to placebo plus ET. Adjuvant ET may include tamoxifen, letrozole, anastrozole, or exemestane for \( \geq 60 \) months with ovarian suppression for premenopausal women at the discretion of the investigator. Adjuvant ET in men will be tamoxifen only. Neoadjuvant therapy is not permitted. Randomization is stratified by menopausal status (men and premenopausal women vs postmenopausal women), prior adjuvant chemotherapy (yes vs no), Prognostic Stage Group (IIA vs IIB), and geographic region (North America/Europe/Australia vs rest of the world). Eligible patients must have tumor tissue from the surgical specimen, adequate bone marrow and organ functions, normal serum electrolytes, QTc interval < 450 msec, and completed and recovered from acute toxicities of adjuvant radiotherapy and/or chemotherapy. The primary endpoint is invasive disease-free survival (per STEEP system; Hudis et al. J Clin Oncol. 2007). Secondary endpoints include recurrence-free survival, distant disease-free survival, overall survival, quality of life, and safety. Global recruitment to EarLEE-2 is ongoing. NCT03081234
Title: PATINA: A randomized open label phase III trial to evaluate the efficacy and safety of palbociclib + anti HER2 therapy + endocrine therapy vs anti HER2 therapy + endocrine therapy after induction treatment for hormone receptor positive, HER2 positive metastatic breast cancer

Otto Metzger-Filho¹, Sumithra Mandrekar¹, Sibylle Loibl², Eva Ciruelos³, Luca Gianni⁵, Elgene Lim⁴, Kathy Miller⁷, Cynthia Huang⁶, Maria Koehler⁵, Prue Francis⁴, Pinuccia Valagussa⁶, Shom Goel¹, Aleix Prat³, Matthew Goetz¹, Sherene Loi⁶, Ian Krop¹, Lisa Carey¹, Jane Lanzillotti¹, Eric Winer¹, Debu Tripathy¹ and Angela DeMichele⁷. ¹Alliance Foundation Trials; ²German Breast Group; ³SOLTI; ⁴Australia & New Zealand Breast Cancer Trials Group; ⁵Fondazione Michelangelo; ⁶Pfizer and ⁷PrECOG.

Body: Background

Pre-clinical data and initial results from clinical studies point to the added benefit of CDK4/6 inhibition when combined with anti-HER2 tx. The current study is designed to evaluate the added benefit of palbociclib when given in combination with anti-HER2 and endocrine tx maintenance in the 1st†line setting of metastatic HER2+HR+ breast cancer.

Trial design

PATINA is an international, open-label, pivotal Phase III study. Primary objective is to demonstrate that the combination of palbociclib with anti-HER2 plus endocrine tx is superior to anti-HER2 plus endocrine tx in prolonging PFS. Sample size is 496 pts. The study starts after completion of 6-8 cycles of chemotherapy-containing anti-HER2 tx for metastatic breast cancer in the 1st line setting. Pts are eligible provided they are without evidence of disease progression by local assessment (i.e. CR, PR or SD). To account for the need for less intense tx regimens for a subset of pts diagnosed with HER2+ER+ disease, clinicians may recommend the combination of trastuzumab with either a taxane or vinorelbine prior to study initiation. Clinicians might also choose a non-pertuzumab option for pts previously treated with pertuzumab in the neo(adjuvant) setting. Secondary objectives include measures of tumor control (OR, CBR, DOR), OS, safety and QOL. The translational science main objective is to compare PFS estimates according to PIK3CA mutation status assessed by cfDNA analysis. Endocrine tx options are AI or fulvestrant. Premenopausal pts must receive ovarian suppression. The study has a 90% power to detect a hazard ratio of 0.667 in favor of the palbociclib arm. Pts approached to participate in AFT-38 will be asked to indicate on the informed consent forms whether remaining biospecimens and clinical data from the control arm of the study can be shared with the Mastering Breast Cancer (MBC) Initiative. The overarching purpose of the MBC is to create a mechanism for understanding the natural history of metastatic breast cancer by cataloguing longitudinally studied tumor-specific markers and treatment effects. ClinicalTrials.gov Identifier: NCT02947685
**Title:** PALLAS: PALbociclib CoLlaborative Adjuvant Study: A randomized phase 3 trial of palbociclib with standard adjuvant endocrine therapy versus standard adjuvant endocrine therapy alone for HR+/HER2- early breast cancer

**Body:**

**Background:**

Cell cycle inhibition is a proven target for novel cancer therapeutics. Palbociclib (P) is an orally active inhibitor of CDK4/6, and arrests the cell cycle at the G1-S transition. P in combination with endocrine therapy (ET) has demonstrated efficacy in phase II and III randomized trials for patients with newly diagnosed and recurrent hormone receptor positive/HER2 negative (HR+/HER2-) metastatic breast cancer (MBC), and is approved in these settings. Given confirmed benefits of P and ET for MBC, the PALLAS study was designed to determine if the addition of P to adjuvant ET improves outcomes over ET alone in HR+/HER2- early breast cancer.

**Trial Design:**

PALLAS is an international open-label phase III trial randomizing (1:1) patients (pts) to 2 years of P (125 mg daily, 21 days on 7 days off in a 28-day cycle) combined with at least 5 years of provider choice ET (AI, tamoxifen, +/- LHRH agonist), versus ET alone. The primary objective of the study is to compare invasive disease-free survival (iDFS) for the combination of P and ET, versus ET alone. Secondary objectives include comparison of iDFS excluding cancer of non-breast origin, DRFS, LRRFS, OS, as well as safety. The principal objective of the translational investigations is to determine the predictive or prognostic utility of defined genomic subgroups with respect to iDFS and OS. Additional objectives include evaluation of cfDNA and tissue biomarkers predictive of benefit or resistance, pharmacogenomics, adherence, and patient-reported QOL. Eligible pts are pre- or post-menopausal women or men with stage II-III, HR+/HER2- breast cancer. Patients may have already initiated ET, but must be randomized within 12 months of diagnosis and 6 months of initiation of adjuvant ET. Trial sample size is 4600 pts and stage IIA pts will be capped at a total accrual of 1000 pts. Interim analyses for safety, futility/efficacy and sample size re-estimation are planned. PALLAS opened in 9/2015 and accrual is ongoing. Contact information: emayer@partners.org

**Key words:** palbociclib, CDK4/6 inhibition, HR+/HER2- early breast cancer, adjuvant endocrine therapy.
**Title**: A randomized phase II trial of fulvestrant with or without ribociclib after progression on aromatase inhibition plus cyclin-dependent kinase 4/6 inhibition in patients with unresectable or metastatic hormone receptor positive, HER2 negative breast cancer (Maintain trial)

Kevin Kalinsky¹, Prabhjot S Mundi¹, Codruta Chiuzan¹, Melissa K Accordino¹, Meghna S Trivedi¹, Josepha A Sparano², Sun Y Oh³, Amy Tiersten³, Ruth O'Regan³, Francisco J Esteva³, Sarika Jain³, Ingrid A Mayer³, Andres Forero³, Chris Vaklavas³, Lea Baer³, Katherine Crew¹ and Dawn L Hershman¹. ¹Columbia University Medical Center; ²Montefiore Medical Center; ³Mount Sinai Hospital; ⁴University of Wisconsin Carbone Cancer Center; ⁵New York University Cancer Institute; ⁶Northwestern University; ⁷Vanderbilt University Ingram Cancer Center; ⁸University of Alabama at Birmingham Comprehensive Cancer Center and ⁹State University of New York at Stony Brook.

**Body**: Background
Cyclin dependent kinase 4/6 inhibitors (CDK4/6i), including palbociclib and ribociclib (R), have demonstrated remarkable benefit in progression free survival (PFS) in patients (pts) with hormone receptor positive (HR+), HER2- metastatic breast cancer (MBC) when combined with anti-estrogen therapy. Switching between anti-estrogen therapies at disease progression is standard of care in the treatment of HR+ MBC. We evaluate the strategy of switching anti-estrogen therapy to fulvestrant (F) and maintaining CDK4/6 inhibition with R in pts with HR+, HER2- MBC who have progressed on an aromatase inhibitor (AI) + CDK4/6i.

**Trial Design**
Phase II, multi-center, randomized, double-blind, placebo-controlled trial to evaluate F +/- R in pts with HR+, HER2- MBC who have previously progressed on any AI + CDK4/6i. Pts can be screened and registered at two different time points:
Scenario 1: Before receiving any CDK4/6i
Scenario 2: At the time of progression of disease (POD) while being treated with an AI + CDK4/6i
In scenario 1, the study will provide pts with letrozole + R, but pts will not be randomized until they demonstrate POD. At randomization, pts will be assigned 1:1 to either a) F + R or b) F + placebo, with treatment given in 4-week cycles. F will be given as a 500 mg dose intramuscularly every 2 weeks for 3 times and then every 4 weeks, as per standard of care. R or placebo will be given orally at 600 mg daily, 3 weeks on/1 week off. CT scans and bone scan are to be performed prior to every third cycle. Serum and whole blood samples and optional tissue biopsies for biomarker assessment will be performed prior to study treatment (scenario 1), prior to randomization to R +/- F, and when the patient goes off study.

**Biomarker assessment** will include amplification of cyclin D1 and cyclin E, phosphoRb and TK1 expression, Rb1 and p16 loss, and ctDNA for ESR1 and PIK3CA mutations.

**Target Accrual**
132 pts accrued from 11 academic medical centers in the U.S, with a goal of completing accrual in two years (~60 to 72 pts in each scenario).

**Statistical Methods**
Assuming a median PFS of 3.8 months with F alone, we predict that F + R will lead to a median PFS of at least 6.5 months. A one-sided log-rank test with a sample size of N=120 and alpha=0.025, achieves 80% power to detect a difference in PFS of 2.7 months. N=132 pts allows for a 10% drop-out rate.

Contact
Body: Background: Hormone receptor positive breast cancer is the most commonly diagnosed subset of breast cancer (60-65%). Endocrine therapy is effective for this subset of breast cancer, in both the adjuvant and metastatic settings. Despite advances in endocrine therapy, many patients relapse during or after completing adjuvant therapy and metastatic breast cancer remains incurable. Palbociclib is a reversible, oral, small molecule inhibitor of cyclin dependent kinases 4 and 6 (CDK4/6). CDK4 and CDK6 together with cyclin D have important roles in regulation of the G1/S transition via regulation of the phosphorylation state of retinoblastomaprotein (Rb). Palbociclib showed significantly improved progression-free survival taken together with endocrine agents in treatment of metastatic breast cancer. Preclinical data showed that in combination with tamoxifen, palbociclib had synergistic growth inhibitory activity as well as efficacy in a model of acquired tamoxifen resistance. Combining palbociclib with tamoxifen in first line treatment of metastatic hormone receptor positive breast cancer may offers an appealing alternative to other endocrine combinations. Methods: This is a non-randomized, open-label, single-arm, multicenter, phase II study of palbociclib in combination with tamoxifen in patients with hormone receptor positive/HER2 negative advanced breast cancer. The primary objective is to determine the objective response rate (complete or partial response) based on RECIST 1.1 or MDA Criteria (for patients with bone only disease). Secondary objectives are: safety and tolerability, progression-free survival, clinical benefit rate, 2-year overall survival. Correlative objectives will explore alterations in circulating tumor DNA and changes in gene expression pattern at the time of progression. Eligibility criteria: women or men with diagnosis of hormone receptor positive/HER2 negative locally advanced or metastatic breast cancer, not amenable to curative surgery; no prior systemic anti-cancer therapy for advanced hormone receptor positive breast cancer; adequate organ function; pre and post menopausal women are allowed. Drug administration: palbociclib dose will be 125 mg orally once daily on days 1-21 of each 28-day cycle; tamoxifen dose will be 20 mg orally once daily for every day of the 28-day cycle. As of June 2017, the study enrolled 10/71 patients and it is still open to enrollment. NCT 02668666; ocdanciu@uic.edu
Title: Palbociclib after CDK inhibitor and endocrine therapy (PACE): A randomized phase II study of fulvestrant versus palbociclib plus fulvestrant, with and without avelumab, for CDK inhibitor pre-treated HR+/HER2- metastatic breast cancer

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Body: Background:
CDK4/6 inhibition (CDK4/6i) has a significant role in contemporary management of hormone receptor positive/HER2 negative (HR+/HER2-) metastatic breast cancer (MBC). The addition of a CDK4/6i to endocrine therapy (ET) in HR+/HER2- MBC leads to prolongation of progression-free survival in previously untreated and pre-treated HR+/HER2- MBC. Mechanisms of resistance to CDK4/6i are not well described, and it is not known if continuation of CDK4/6i with subsequent lines of ET improves outcomes over ET alone. Further, preclinical data suggest combination therapy with ET, CDK4/6i, and anti-PDL1 may provide synergistic efficacy. The PACE trial was designed to determine the optimal subsequent line of therapy in patients (pts) with HR+ /HER2-MBC that has progressed despite prior CDK4/6 inhibition and endocrine therapy.

Methods:
PACE (NCT03147287) is a multicenter phase II trial randomizing pts 1:2:1 to Arm A: fulvestrant alone (with option for Palbociclib (P) monotherapy crossover at time of progression); Arm B: fulvestrant + P; or Arm C: fulvestrant + P + avelumab. The primary objective is to evaluate progression-free survival (PFS) with the combination of fulvestrant + P vs. fulvestrant alone; secondary objectives include overall response (OR), safety and tolerability, and PFS comparisons of avelumab containing arm vs other arms. Exploratory objectives include assessment of outcomes in predefined molecular subgroups (i.e. ESR mutation, PI3K mutation, and loss of Rb); and comparing OR by RECIST vs irRECIST in the avelumab cohort. Extensive molecular profiling of tissue, ctDNA, and CTCs for markers of response and resistance to therapy is also planned. Eligible pts have HR+/HER2- MBC, with prior response to and subsequent progression on any CDK4/6i and ET, defined as at least 6 months of prior treatment, with confirmed subsequent progression, and no more than one prior P dose reduction for toxicity. Pts may have had 1-2 prior ET (aromatase inhibitor or tamoxifen), and 0-1 prior lines of chemotherapy. A sample size of 220 patients is planned.
Title: SHERBOC: A double-blind, placebo-controlled, phase 2 trial of seribantumab (MM-121) plus fulvestrant in postmenopausal women with hormone receptor-positive, heregulin positive, HER2 negative metastatic breast cancer whose disease progressed after prior systemic therapy

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Body: Background: The receptor tyrosine kinase, HER3 and its ligand, heregulin (HRG), have been implicated in the initiation and progression of multiple cancer types including: breast, lung, and head & neck cancers. Seribantumab is a fully human, monoclonal IgG2 antibody that binds to the ligand-binding domain of HER3 and inhibits HRG-mediated signaling. Previously, seribantumab was tested in combination with exemestane in a placebo-controlled, Phase 2 study in post-menopausal women with ER/PR+, HER2 negative metastatic breast cancer (mBC). Although the trial failed to meet its primary efficacy objective of a 50% reduction in hazard ratio in the seribantumab/exemestane treatment vs. the placebo/exemestane control group, a positive trend in PFS and a statistically significant improvement in median OS was observed in patients in the seribantumab/exemestane treatment group. Seribantumab has also been tested in three randomized Phase 2 studies adding to standard of care (SOC) in non-small cell lung, ER/PR+ mBC, and platinum resistant/refractory ovarian cancer. These studies were retrospectively analyzed to determine correlation between HRG mRNA levels in tumor tissue and PFS. In each of these studies, the presence of tumor cell HRG mRNA was prognostic for shortened PFS with SOC treatment. Further, the addition of seribantumab to SOC therapy improved PFS for patients with HRG+ tumors. These data support the hypothesis that HRG expression may define a drug tolerant cancer cell phenotype characterized by poor response to multiple classes of cytotoxic and targeted therapies, including aromatase inhibitors and SERDs. Additionally, blockade of HRG-induced HER3 signaling by seribantumab may counter such protective effects of HRG on cancer cells, with the potential for improved outcomes in HRG+ patients. It is estimated that ~45% of hormone-receptor positive, HER2 negative advanced breast cancers are HRG+ and that HRG expression may contribute to accelerated clinical progression observed in this subset of patients.

Trial design: In the upcoming randomized, double-blinded, multi-center, Phase 2 study, ER/PR receptor-positive, HER2 negative mBC patients with HRG+ tumors will be prospectively selected using a HRG RNA in situ hybridization assay. Approximately 200 women will be screened to enroll 80 HRG+ subjects. Eligible subjects will be randomized in a 1:1 ratio to receive seribantumab/fulvestrant or placebo/fulvestrant until investigator-assessed disease progression or unacceptable toxicity, whichever comes first. Subjects will have progressed on one or two prior hormonal therapies, one of which must have been a CDKi-containing regimen. The goal of this study is to determine if the combination of seribantumab + fulvestrant is more effective than placebo + fulvestrant based on PFS (primary end point) in HRG positive subjects. Secondary endpoints include OS, objective response rate, and time to progression. Safety will also be assessed. Enrollment is expected to begin in 2017 at approximately 80 sites globally.
Title: A phase Ib trial of xentuzumab and abemaciclib in patients with locally advanced or metastatic solid tumors, including hormone receptor-positive, HER2-negative breast cancer (plus endocrine therapy)

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Body: Background: Resistance to endocrine therapy remains an important clinical problem in hormone receptor-positive (HR+), HER2-negative (HER2-) breast cancer (BC), necessitating alternative treatment options. The insulin-like growth factor (IGF) axis and cyclin D-cyclin-dependent kinase (CDK) 4/6-retinoblastoma pathway have been implicated in the pathogenesis and resistance mechanisms of a variety of cancers, including BC. Binding of IGF-I and -II to the IGF receptor results in upregulation of cyclin D1, and subsequent progression through the cell cycle, thus providing rationale for the simultaneous inhibition of IGF-I and -II and CDK4/6. This Phase Ib trial assesses the maximum-tolerated dose (MTD)/recommended phase II dose (RP2D), safety and preliminary efficacy of the IGF-ligand-neutralizing antibody, xentuzumab, in combination with abemaciclib, a selective, small-molecule inhibitor of both CDK4 and 6, in patients (pts) with solid tumors. The trial includes four dose finding cohorts followed by two expansion cohorts. Only those cohorts that will include pts with postmenopausal HR+, HER2- BC will be presented here.

Trial design: In this phase Ib multicenter, non-randomized, open-label, dose escalation trial (BI 1280.18 [NCT03099174]), the key aims in the BC cohorts (Cohorts B–D, F) are to define the MTD or recommended phase 2 dose (RP2D), and to evaluate the preliminary efficacy, safety and tolerability of xentuzumab plus abemaciclib in combination with endocrine therapies. Eligible pts include adults ≥18 yrs (≥20 for Japan), with measurable or evaluable disease, adequate organ function, ECOG PS ≤1, and postmenopausal locally advanced or metastatic HR+, HER2- BC (Cohorts B–D, F). CDK4/6 inhibitor-naïve pts (Cohorts B–D) and pts who have received prior CDK4/6 inhibitors (palbociclib or ribociclib) plus aromatase inhibitors (Cohort F) are included. The MTD/RP2D of xentuzumab plus abemaciclib to be used in Cohorts B–D will be established in pts with solid tumors (Cohort A) who will receive xentuzumab (starting dose 1000mg weekly iv) plus abemaciclib (starting dose 150mg every 12 hours). CDK4/6 inhibitor-naïve pts with BC will receive xentuzumab plus abemaciclib at the RP2D determined in Cohort A in combination with letrozole (2.5mg/day; Cohort B), anastrozole (1mg/day; Cohort C), or fulvestrant (500mg/month; Cohort D). CDK4/6 inhibitor pre-treated pts with BC (Cohort F) will receive xentuzumab plus abemaciclib and fulvestrant at the RP2D determined in Cohort D. Primary endpoints in the BC cohorts are the MTD and/or RP2D of xentuzumab plus abemaciclib in combination with endocrine therapies, and the objective response (OR) in CDK4/6 inhibitor pre-treated pts with advanced BC (Cohort F); disease control (DC), duration of DC, time to OR, duration of OR, and progression-free survival (PFS) in Cohort F are secondary endpoints. Additionally, PK outcomes, safety and tolerability will be assessed in all cohorts. This study will be conducted in the US, Europe and Japan. Pt screening started in May 2017. Target enrolment is ~88 pts, including ~56 pts with advanced HR+, HER2- BC, of whom ~20 had previously been treated with CDK 4/6 inhibitors.
Full Title: A randomized, open-label, multi-center, phase II study to compare the efficacy and tolerability of atorvastatin 40 mg in addition to endocrine treatment in patients with estrogen receptor (ER) positive advanced breast cancer with focus on mechanisms of resistance.

Background: The majority of metastatic breast cancer patients progress during endocrine therapy and eventually become resistant to treatment. Understanding how metastatic cancer cells adapt to different therapies is key for the development of improved treatment regimens. The effectiveness of endocrine therapy in ER+ tumors may be influenced by cholesterol through the cholesterol metabolite oxysterol 27-hydroxycholesterol, which acts as an ER ligand, harboring the ability to regulate ER-dependent tumor growth. Statin-mediated inhibition of the cholesterol pathway has been demonstrated to induce anti-neoplastic effects in both breast cancer cells and human breast cancer. Hence the goal of this study is to both understand the mechanisms of resistance to endocrine treatment and test the hypothesis that addition of statins will enhance the efficacy of endocrine treatment.

Trial Design: A multi-center randomized, open-labelled, phase II trial in the first and second line metastatic treatment setting, comparing standard endocrine treatment (letrozole) with letrozole +/- atorvastatin (1:1). Upon progression in the first line setting, and as part of the translational studies of mechanisms of resistance to endocrine therapy, the patients receive second line endocrine treatment using fulvestrant.

Eligibility criteria: 1) Patients diagnosed with ER positive/HER2 negative metastatic breast cancer, including locally advanced stage IV disease, requiring systemic endocrine treatment. 2) No Previous treatment for metastatic breast cancer, unless being considered for direct entry to the second part of the study with fulvestrant.

Specific aims: To test the clinical efficacy of adding statins to endocrine treatment in advanced breast cancer. Primary endpoint: Clinical benefit rate, defined as the proportion of all randomly assigned patients who have the best overall response; complete response, partial response, or stable disease for at least 24 weeks following first-line letrozole treatment alone or in combination with atorvastatin. Translational endpoint: To elucidate mechanisms of resistance to endocrine treatment alone or in combination with statins in ER+ metastatic breast cancer.

Statistical Methods: The primary endpoint of clinical benefit rate will be compared in the two groups using a logistic regression model where the odds ratios and associated 95% CIs and p-values will be reported. The secondary endpoint, progression-free-survival, will be analyzed in crude analysis using the Kaplan-Meier and Log-Rank test as well as the Cox regression hazards analysis with the latter allowing for confounder-controlled multivariate analysis.

Present accrual and target accrual: The trial started recruiting as of October 10, 2016. The target accrual is 126 patients, whereof 17 are presently included in the trial.

Contact information for people with a specific interest in the trial:
Signe.Borgquist@med.lu.se
2017 San Antonio Breast Cancer Symposium

Publication Number: OT3-06-04

Title: A randomized phase II study of neoadjuvant panitumumab/carboplatin/paclitaxel (PaCT) versus carboplatin/paclitaxel (CT) followed by adriamycin and cyclophosphamide (AC) for newly diagnosed primary triple-negative inflammatory breast cancer (TNIBC)

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Body: Primary Objective: To determine pathological complete response (pCR) rate in patients with primary TNIBC treated with PaCT in comparison with CT, followed by AC. To explore if the pCR rate correlates with reduced nodal expression status; and with arginine methylation status of epidermal growth factor receptor (EGFR). We will identify molecular biomarkers predictive of the pCR rate by analysis of multiplexed immunohistochemical (IHC) staining, identify molecular biomarkers predictive of the pCR rate by genomic and proteomic analysis, and determine whether the inhibition of the EGFR pathway down regulates the COX-2 pathway and mesenchymal marker.

Background: EGFR is overexpressed in triple negative breast cancer (TNBC) and inflammatory breast cancer (IBC). Therefore, EGFR targeted therapy may have a promising role in TNBC and IBC. A study showed that EGFR-targeted therapy may enhance the initial chemosensitivity of TNBC cells. Panitumumab blocks epidermal growth factor ligands and transforming growth factor EGFα (TGFα) binding to EGFR, inhibits tumor growth, and elicits both tumor regression and eradication of established tumors in murine xenograft tumor models. Panitumumab, a fully humanized anti-EGFR antibody, has been shown to be active in a breast cancer preclinical model using human breast cancer cell line MDA-MB-468, which has been shown to overexpress EGFR by both IHC and fluorescence in situ hybridization (FISH). Furthermore, EGFR tyrosine kinase inhibitors such as erlotinib have antitumor activity against human IBC cell lines. Thus, EGFR targeted therapy may have a promising role in TNBC and IBC.

Study Design: In this open label randomized phase II trial, up to 72 patients with primary IBC, have no HER2 overexpression, and have <10% expression of ER and PgR, who also meet other criteria will be randomized to PaCT arm - receiving panitumumab single agent in window study and 4 cycles PaCT, or CT arm - receiving 4 cycles of CT. All patients will receive 4 cycles of AC before surgery.

Statistical Considerations: A sample size of 36 patients per arm will achieve 84% power to detect a difference of 0.24 in pCR rate between 0.2 in the CT arm and 0.44 in the PaCT arm with a type I error rate of 10% using one-sided Z test. Based on historical data, we expect that the pCR rate of a PaCT regimen to achieve 24% additional efficacy compared with the CT regimen.

Status of the study: Activation date: Oct. 2016. So far 6 patients have been enrolled. Enrollment continues.

Sponsor: Amgen.
State of Texas appropriation for rare and aggressive breast cancer research.
NIH grant 1R01CA205043-01A1
2017 San Antonio Breast Cancer Symposium

Publication Number: OT3-06-05

Title: A phase Ib/II trial of coPANlisib in combination with tratuzumab in pretreated recurrent or metastatic HER2-positive breast cancer “PantHER”

Niamh M Keegan1,2,7, Janice Walshe3, Giuseppe Gullo3, John Kennedy4, Kyran Bulger5, Catherine M Kelly6, John Crown3, Sinead Toomey1, Keith Egan1, Jennifer Kerr2, Mark Given2, Andrés Hernando8, Ausra Teiserskiene8, Liam Grogan2, Oscar Breathnach2, Patrick G Morris2,7,8, Maccon Keane9 and Bryan T Hennessy1,2,7,8. 1RCSI Molecular Medicine, Dublin, Ireland; 2Beaumont Hospital, Dublin, Ireland; 3St Vincent's University Hospital, Dublin, Ireland; 4St James's Hospital, Dublin, Ireland; 5Midland Regional Hospital at Tullamore, Tullamore, Ireland; 6Mater Misericordiae University Hospital, Dublin, Ireland; 7Cancer Clinical Trials & Research Unit, Beaumont Hospital, Dublin, Ireland; 8Cancer Trials Ireland, Dublin, Ireland and 9Galway University Hospital, Galway, Ireland.

Body: Background
The phosphoinositide 3 kinase (PI3K) pathway is important in the oncogenic function of HER2. Aberrant activation of PI3K is implicated in resistance to trastuzumab and other HER2-targeted therapies and is frequent, with up to 22% of HER2 positive breast cancer having a PIK3CA mutation. Copanlisib is a pan-class 1 PI3K inhibitor that shows particular activity against PI3Kα, the isoform encoded by the PIK3CA gene. Copanlisib has been shown to re-sensitise trastuzumab resistant cell lines to trastuzumab with synergism seen in some cell lines between copanlisib and HER2 targeted therapy.

Trial design
The study is a phase Ib/II open label, single arm adaptive, multi-centre trial of copanlisib in combination with trastuzumab. Eligible patients are treated with a dose escalation schedule of copanlisib IV on Days 1, 8 and 15 of a 28 day cycle with trastuzumab 2 mg/kg weekly (loading dose of 4 mg/kg in cycle 1). The phase II dose will be based on the maximum tolerated dose (MTD) established in Phase Ib. Patients are treated until radiologic or symptomatic progression, unacceptable toxicity, consent withdrawal or physician’s decision.

Eligibility criteria
Eligible patients must have recurrent incurable or metastatic HER2-positive breast cancer that has progressed on at least one prior line of trastuzumab or T-DM1-based treatment regimen in this setting. Patients with treated and controlled brain metastases are eligible. Participants must have adequate organ function and ECOG PS ≤ 2. Patients recruited for the Phase II part of the study must have a PIK3CA mutation. Patients with uncontrolled arterial hypertension, uncontrolled diabetes or recent clinically serious infections are excluded.

Specific aims
The primary end point for the phase Ib part of this study is to determine the MTD for the combination. For the phase II study is anti-tumour efficacy, measured by Clinical Benefit Rate (CBR).

Secondary end points are evaluation of safety and tolerability, progression-free survival, time to treatment failure, duration of response and overall survival. Incorporated translational endpoints include examination of molecular tumor adaptation in tissue and blood. Given the role of PI3K in cellular glucose metabolism, an additional exploratory objective is to determine if quantitative reduction in metabolic signal on Positron Emission Tomography-Computed Tomography (PET-CT) is predictive of benefit from therapy.

Statistical methods
To establish the MTD, we use a modified 3+3 design where 3 additional patients will be accrued even if the first 3 patients accrued experience no dose limiting toxicities (DLT) in sequential cohorts for a planned 12 patients. To determine the CBR, a one sample exact binomial test with a one sided significance level of 5%, 19 evaluable patients will provide >80% power to detect a difference between the null hypothesis proportion of 30% for CBR versus the alternative hypothesis proportion of 65%.

Present accrual and target accrual
There are 9 patients recruited so far to the phase Ib part of this study. Target accrual is 12 and for phase II is 19 patients.

Contact information for people with a specific interest in the trial
Prof Bryan Hennessy, Beaumont Hospital, Dublin Ireland
Funded by Bayer
Title: A phase I/II study of preoperative letrozole, everolimus, and TRC105 in women with newly diagnosed local or locally advanced potentially resectable hormone-Receptor positive and Her2 negative breast cancer

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Body: Hypothesis: Pharmacologic inhibition of angiogenesis and mTOR pathway blockade will enhance the efficacy of aromatase inhibitors in the neoadjuvant setting in women with hormone receptor-positive and Her2-negative (HR+/Her2-) breast cancer.

Background: In non-metastatic HR+/Her2- breast cancer, downstaging to stage I or 0 in the neoadjuvant setting has been associated with favorable prognosis. With chemotherapy yielding similar benefits with endocrine therapy, aromatase inhibitors have become the agents of choice in postmenopausal women. The elucidation of pathways that are operational in luminal breast cancer and compromise the efficacy of hormonal therapy along with preclinical and clinical evidence of successful combinations of endocrine therapy with targeted agents set the stage for more rational, effective, and equally well tolerated regimens. Proangiogenic signaling has been strongly associated with accelerated hormone-independent growth and resistance to endocrine therapies. Breast cancer cells with upregulated PI3K/AKT/mTOR pathway are resistant to hormonal therapy and this resistance has been restored by everolimus. Inhibiting mTOR, a downstream signaling node where multiple pathways converge has the potential to abrogate primary and escape signaling cascades that mediate resistance to endocrine therapies.

Study Design: Phase I dose escalation: follows a 3+3 design. During the phase I, the Maximum Tolerated Dose (MTD) and Recommended Phase 2 Dose (RP2D) will be determined. Letrozole will be kept the same for all cohorts (2.5 mg PO qd). Everolimus will be escalated from 5 mg (cohort 1) to 10 mg PO qd (cohort 2). TRC105 will be 15 mg/kg q2 weeks (cohorts 1 and 2). Premenopausal women at the time of enrollment will receive goserelin 3.6 mg SC q4 weeks to achieve ovarian suppression. Duration of treatment is 24 weeks.

Phase II dose expansion: will be initiated once the MTD and RP2D have been determined. Phase II follows a Gehan's two-stage design: 10 patients will enroll first, and if no patient has achieved downstaging, the study will close since it is unlikely (p=0.1) that 0/10 responses would occur if true response rate were >20%. If at least one patient has downstaging, 10 additional patients will enroll (total, 20).

Primary Objectives
Phase I: Determine the tolerability and feasibility of letrozole, everolimus, and TRC105 in women with newly diagnosed stage 2 and 3 HR+/Her2- breast cancer.

Phase II: in the same patient population determine the efficacy, pharmacokinetic, and pharmacodynamic parameters of the combination.

Correlative Studies: Immunohistochemistry for pAKT and PTEN, CD105, CD31/CD34, NG2, MCAM/CD146 on diagnostic tissue, research biopsy, and final surgical specimen. Surgical specimens that meet criteria for next-generation sequencing will undergo ribosome profiling

Key Eligibility Criteria: Recent diagnosis of HR+Her2- breast cancer
Stage 2 and 3 breast cancer
Any histological grade
No prior treatment specific for breast cancer
Pre- and perimenopausal women are eligible if ovarian suppression is achieved with goserelin.

Current Status: Cohort 1 of the Phase 1 complete. Cohort 2 of Phase 1 actively enrolling.
A phase I/II dose escalation and expansion study to investigate the safety, pharmacokinetics, pharmacodynamics and clinical activity of GSK525762 in combination with fulvestrant in subjects with ER+ breast cancer

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Background:
Advanced or metastatic ER+BC (estrogen receptor positive breast cancer) is an incurable illness that will prove fatal for most afflicted women. Current standards of care include endocrine, targeted, and chemotherapy. Preclinical data suggest that altering the expression of the estrogen receptor (ER) as well as other ER-responsive genes may provide therapeutic benefit for women for whom endocrine therapy alone has proven inadequate. The bromodomain (BRD) and extra-terminal (BET) family of proteins (BRD2, BRD3, BRD4 and BRDT) bind to acetyl-histone residues and epigenetically control transcription of genes driving cell survival and proliferation. BET proteins have been implicated in carcinogenesis and treatment resistance in multiple tumors including ER+BC, and are a novel target for therapy in breast cancer. GSK525762 is a pan-BET inhibitor that has shown strong synergistic activity with fulvestrant in killing ER+BC cells in vitro and in xenograft models. The combination of BET agents with endocrine therapy may provide therapeutic benefit and restore sensitivity to ER targeting agents like fulvestrant.

Trial Design & Specific Aims:
This study is a Phase I/II dose-escalation, expansion (Phase I) and randomized control (Phase II) study with oral administration of GSK525762 in combination with fulvestrant in advanced or metastatic ER+BC subjects, whose disease has progressed on prior treatment with at least one line of endocrine therapy.
Phase I of the study is designed as parallel single arms to determine a recommended Phase 2 dose (RP2D) based on safety, tolerability, pharmacokinetic, and efficacy profiles in two distinct populations of ER+ breast cancer:
Subjects with disease that relapsed during treatment or within 12 months of adjuvant therapy with an AI, OR disease that progressed during treatment with an AI for advanced/metastatic disease.
OR
Subjects with disease that progressed during treatment with the combination of a CDK4/6 inhibitor plus letrozole for advanced or metastatic disease.
Phase II of the study is a randomized, double-blind, placebo-controlled cohort, designed to evaluate the efficacy of the combination.

Key Eligibility Criteria: Patients must have received <3 lines of systemic anti-cancer therapy (≤1 line of chemo), measurable disease, and PS 0-1.

Statistical Methods: A modified toxicity probability interval (mTPI) design will be used to monitor safety. A Bayesian adaptive design will be used to evaluate efficacy in Phase 1.

Present and Target Accrual: Target enrolment will be ~300 subjects across ~50 sites worldwide. To date, 2 subjects have been enrolled.
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NCT02964507
Funding: GSK
2017 San Antonio Breast Cancer Symposium

Publication Number: OT3-07-01

Title: Update of the randomized, non-inferiority LORD trial testing safety of active surveillance for women with screen-detected low risk ductal carcinoma in situ (EORTC-1401-BCG/BOOG 2014-04, DCIS)

Jelle Wesseling¹, Lotte E Elshof¹, Konstantinos Tryfonidis², Coralie Poncet², Kim Aalders³, Elise van Leeuwen-Stok³, Victoria Skinner¹, Claudette Loo¹, Gonneke Winter-Warnars¹, Eveline Bleiker¹, Valesca Retèl¹, Ruud Pijnappel⁴,⁵, Nina Bijker⁶, Emiel Rutgers¹ and Frederieke van Duijnhoven¹. ¹Netherlands Cancer Institute - Antoni van Leeuwenhoek Hospital, Amsterdam, Netherlands; ²European Organization for Research and Treatment of Cancer (EORTC), Brussels, Belgium; ³Dutch Breast Cancer Research Group (BOOG), Amsterdam, Netherlands; ⁴University Medical Center Utrecht, Utrecht, Netherlands; ⁵Dutch Reference Center for Screening, Nijmegen, Netherlands and ⁶Academic Medical Center, Amsterdam, Netherlands.

Body: The introduction of population-based breast cancer screening and implementation of digital mammography have led to an increased incidence of ductal carcinoma in situ (DCIS) without a decrease in the incidence of advanced breast cancer. This suggests DCIS overdiagnosis exists.

We hypothesize that asymptomatic, low-grade DCIS can safely be managed by active surveillance. If progression to invasive breast cancer would still occur, this will be low-grade and hormone receptor positive with excellent survival rates. Also, breast-conserving treatment will still be an option, if no prior radiotherapy has been applied. Management by active surveillance also may save many low-grade DCIS patients intensive treatment.

Therefore, we will compare active surveillance with conventional treatment, being either mastectomy, wide local excision (WLE) only, or WLE plus radiotherapy, possibly followed by hormonal therapy for primary low-grade DCIS. For this, we conduct a phase III, open-label, non-inferiority, multi-center, randomized clinical trial sponsored by the European Organization for Research and Treatment of Cancer (EORTC-1401-BCG). The Dutch Centers are coordinated by the Dutch Breast Cancer Research Group (BOOG) (BOOG 2014-04). This trial is developed and implemented in close collaboration with patient advocates.

Randomization will be in a 1:1 ratio among one of the following arms: (1) active surveillance or (2) standard treatment per local policy. In total, 1,240 women (≥ 45 years) will be included without prior breast cancer, but with asymptomatic, pure, low-grade DCIS, based on a minimum of tissue harvested by biopsy from calcifications detected by population-based or opportunistic screening. Assuming 25% of randomized women qualified to enroll in the study will drop out or will be excluded from per protocol evaluation, at least 1,240 women need to be randomized to obtain the 930 patients required for the evaluation of the primary endpoint. The same follow-up scheme will be applied in both study arms, i.e. annual mammography for a period of 10 years. The primary end-point is ipsilaterial invasive breast tumor-free rate at 10 years. Secondary end-points are among others: overall survival, breast cancer-specific survival, mastectomy rate, patient reported outcomes and cost-effectiveness. Accrual has started in the Netherlands in February 2017 and will start internationally in over 30 centers shortly.

Acknowledgements: This trial is funded by Pink Ribbon Netherlands, the Dutch Cancer Society and Dutch Cancer Society/Alpe d’HuZes, and Cancer Research UK.
Body: Background: LORIS is a multi-centre, randomised (1:1) controlled trial of Surgery v Active Monitoring with annual mammography in patients with low risk ductal carcinoma in situ (DCIS). During a 2 year Feasibility Study potential patients were invited to complete the Clinical Trials Questionnaire (CTQ) and participate in a semi structured telephone interview about the verbal, written and DVD based trial information. The DVD was produced to complement the patient information sheet (PIS) and incorporates simple graphics and a Q&A session with women asking the Chief Investigator questions about the trial.

Aims: To examine the reasons for trial participation/rejection and obtain feedback about the clarity, timing and usefulness of the PIS and DVD in order to identify potential communication drivers and barriers to trial recruitment.

Methods: Participants completed the CTQ prior to randomisation and with their consent were contacted following randomisation for an interview. Women declining the trial were issued with an optional pack containing the CTQ and the researchers' contact details if they wanted an interview. The CTQ comprises 16 reasons that might influence a decision to either accept or decline a trial. For each statement participants register their agreement or disagreement on a scale of 1 (strongly agree) to 5 (strongly disagree) and indicate the most important reason for their decision. Interviews explored factors such as, attitudes about randomisation, and usefulness of the trial information provided.

Results: 41 patients were randomised during feasibility; 20 surgery, 21 active monitoring, 16 patients declined the trial. 40/41 (98%) acceptors and 9/16 (56%) decliners completed the CTQ. The main reason for joining LORIS was: “I thought the trial offered the best treatment available” 13/40 (32%) and for declining the trial was “The idea of randomisation worried me” (4/9; 44%). 35 interviews were conducted (31/41 (76%) accepted and 4/16 (25%) declined LORIS). At interview acceptors commented that the PIS was very useful and clear (84%; 26/31 & 90%; 28/31, respectively). 74% (23/31) of women who joined LORIS watched the DVD and the majority (19/23; 83%) found it “very useful” and 22 (22/23; 96%) “very easy to understand”. A third of women (10/31) said the PIS and the DVD helped them decide to participate in LORIS. Women who declined the trial had clear treatment preferences; 2/4 did not watch the DVD. Three quarters of women interviewed (19/25) watched the DVD with family members/friends and found it reassuring. One commented it was “Put in words you can understand and not be baffled by”. The most popular aspect was the Q&A session (13/25; 52%).

Conclusions: The LORIS DVD was a useful, easy to understand recruitment tool, complementing the PIS. Many women felt reassured that the content was consistent with, and added to that provided by healthcare professionals. Opinions of family and friends, worries about randomisation and personal preferences exert an influence of those declining these types of trial.

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Title: Disrupting ErbB2 tumor progression via Jak1/Stat3 signaling

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Body: The majority of breast cancers are driven by hormones and growth factors, a subset of which overexpress the human epidermal growth factor ErbB2/Her2. While these cancers overexpress ErbB2 they also rely on IL-6 signaling, exhibiting high activity of Signal Transducer and Activator of Transcription 3 (STAT3). A notorious transcription factor, active STAT3 has been tightly linked to aggressive disease, promoting a metastatic phenotype in these breast cancers. While current treatments against ErbB2 are effective, resistance to targeted therapies and increased metastasis are concerning. Understanding the mechanisms by which inflammatory cytokines contribute to aggressive behavior of breast cancer cells is key. We recently demonstrated that Janus kinase 1 (JAK1) is the essential kinase for mediating inflammatory cytokine signaling in the mammary gland. Ablation of JAK1 in mammary epithelium of mice dramatically prevented STAT3 activation downstream of IL-6 class ligands such as Oncostatin M (OSM) and Leukemia inhibitory factor (LIF).

While past studies have shown that STAT3 deficient mammary tumors have a reduced propensity to metastasize in an ErbB2-driven breast cancer model, current attempts at directly targeting STAT3 with pharmacological agents have been met with limited success. Based on our evidence that JAK1 specifically mediates effects of inflammatory cytokines to activate STAT3 in the mammary gland, we hypothesize that JAK1 is essential for the persistent activation of STAT3 during the progression of ErbB2-associated mammary cancer.

Methods: Mice with the oncogenic ERBB2 tumors (n=20) were compared with those genetically lacking JAK1 (n=14) for tumor onset and lung metastasis. Primary cell lines were generated from control tumors and were tested by RNA-Sequencing, protein and immunostain analysis, and transplant studies to identify growth variations in established tumor cells.

Results: Control and experimental females lacking JAK1 prior to tumor onset display similar tumor latency, suggesting that signaling through JAK1 is not critical for cancer initiation. However, we observed a significant decrease in engraftment and growth of neoplastic cells when Jak1 was deleted in cancer cells and transplanted into wildtype recipient mice. Molecular studies using murine and human cancer cell lines with and without JAK1 revealed that this kinase is essential for activation of STAT3 as well as STAT1 and STAT6. Hence, other Janus kinases expressed in mammary cancer cells (i.e., JAK2 and Tyk2) are unable to compensate for the loss of JAK1. Using RNA-sequencing, we identified several candidate genes controlled by JAK1 and known to correlate with active STAT3.

Conclusions: Our study provides experimental evidence that JAK1 is an essential mediator of inflammatory cytokine signaling in breast cancer cells, and this tyrosine kinase plays a critical role in mammary cancer progression. In contrast to the current paradigm, JAK1 has nonredundant functions for activation of STAT1, 3, and 6 that are persistently tyrosine phosphorylated in mammary cancer cells. Lack of JAK1 and oncogenic STAT3 inhibits expression of other tumor susceptibility genes. Our collective findings suggest that JAK1 is a rational target to prevent breast cancer progression.
Title: Domain-targeted anti-Trop-2 monoclonal antibodies show key therapeutic efficacy against breast cancer

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Body: Trop-2 is a calcium signal transducer that is is overexpressed by most primary breast cancers. The Trop-2 extracellular domain contains an N-terminal cysteine-rich globular domain followed by a stem-like cysteine-less region that is juxtaposed to the transmembrane domain. Trop-2 interacts with tight-junction proteins, which may severely affect accessibility by therapeutic monoclonal antibodies (mAb) directed against the immunodominant epitope between the globular and stem regions. This is thought to account for limited or no therapeutic efficacy of such mAb. In order to fully exploit the potential of anti-Trop-2 immunotherapy we generated novel anti-Trop-2 mAb designed to be specific for the globular and the stem regions.

In vivo, naked anti-globular OX-G64 and anti-stem OX-S55 mAb were most effective in inhibiting the growth of breast cancer xenotransplants in nude mice. NSG mice and in vitro mechanism profiling indicated efficient ADCC. Efficacy was also shown in isolated-cell models of metastatic dissemination. Live-cell imaging, in vitro signal transduction and cancer cell growth inhibition assays revealed that anti-Trop-2 mAb also signal at the cell membrane and directly inhibit tumor growth. We discovered that Trop-2 signaling triggers a ubiquitous multi-molecular complex at the cell membrane, which includes Na+/K+-ATPase, PKCa, CD9 and ezrin. Trop-2-driven Ca2+ influx and remodeling of cell cytoskeleton then leads to growth stimulation of cancer cells, through induction of Akt, MEK and ERK downstream effector kinases.

In a previous study on a 702 consecutive breast cancer case series, we showed that the membrane-localized, fully mature form of Trop-2 is specifically associated with worse patient survival. Here we show that joint assessment of the Trop-2 signaling determinants discriminates with much higher efficiency tumor cases with worse disease outcome. The highest clinical impact was reached in triple-negative breast cancers (TNBC). Trop-2-based multiparametric analysis was able to discriminate TNBC subgroups where all patients showed metastatic relapse from those where disease recurrence never occurred, for unprecedented clinical impact.
The effect of combination therapy in targeting dual signalling pathways in the treatment of breast cancer

Anchala I Kuruppu¹, Micheal J Stocks¹ and Tracey D Bradshaw¹. ¹University of Nottingham, Nottingham, United Kingdom.

Body: Breast cancer is a complex multi-factorial disease which involves many activated molecular pathways demonstrating cross talk. This in turn increases cell growth and survival. Although there have been many novel therapies reaching the clinic, the success rate is low. This could be due to cancer cells using compensatory pathways to thrive and gain acquired resistance to these agents. In order to address this issue, a combination strategy could be used that targets multiple pathways. Thus, we tested the effect of novel dual PI3K/AKT/mTOR inhibitors and Gefitinib (EGFR inhibitor) and 5F 203 (an Aryl hydrocarbon Receptor (AhR) ligand) in a panel of breast cancer cell lines.

Two novel analogues (MS-74 and MS-76) of PF-05212384/PKI-587 (MS-73) which is a dual PI3K/AKT/mTOR inhibitor, were implemented to increase solubility. MDA-MB 468 which is a PTEN deficient cell line was the most sensitive towards all three agents. MS-73 (GI₅₀=0.015µM ± 0.001), MS-74 (GI₅₀=0.233µM ± 0.009) and MS-76 (GI₅₀=1.31µM ± 0.41). MDA–MB 468 cells portrayed a very low survival fraction in clonogenic assays for MS-74 (P<0.0001). MS-74 also showed an extremely significant pre-G1 accumulation against MDA-MB 468 cells compared to control at 24 and 72 h for 0.15 µM and 1.5 µM (P<0.0001) which was indicative of apoptosis. Further, both MS-74 and MS-76 evoked G1 accumulations that was followed by decreased S and G2/M phases in MDA-MB 468 cells with the highest concentration tested- 1.5 µM (P<0.0001). MS-74 caused apoptosis with 0.15 µM (P<0.05) and 1.5 µM (P<0.01) at 24 h in MDA-MB 468 cells. However, no significant apoptosis was observed following treatment of cells with 0.15 µM at 72 h but only with 1.5 µM (P<0.0001) at the same time point. MS-76 did not induce prominent apoptosis in this cell line. Western blot analysis demonstrated that both MS-74 and MS-76 downregulated phosphorylated (p)-4E-BP1 (Ser65) and p-eIF4E (Ser209) extremely significantly (P<0.0001). These results suggest that the novel analogues, especially MS-74 could be very effective in breast tumours lacking PTEN.

AhR is highly expressed in the breast and shown to influence cell proliferation by interacting with pathways such as EGFR, thus the combination effect of Gefitinib and 5F 203 was tested. We found that the HER2+ SKBR3 cell lines was the most sensitive to Gefitinib (GI₅₀=0.94µM ± 0.85) and 5F 203 (GI₅₀=20.41nM ± 0.90) alone. Interestingly, both agents in combination showed a combination index of 0.64 ± 0.08 in SKBR3 cells, which is indicative of synergism according to the Chou and Talalay theorem. SKBR3 colony formation was reduced with the agent combination compared to control (P<0.0001). At 24 and 72 h the agent combination showed an extremely significant total apoptotic population compared to both agents alone and SKBR3 control (P<0.0001). It also showed an enhanced positive population for DNA double strand breaks at 24 and 72 h relative to SKBR3 control (P<0.0001). Further, the agent combination, downregulated c-MET protein expression (P<0.0001) which may prevent upregulation of compensatory pathways. Thus, this combination may offer a potential novel treatment for HER2+ breast cancer. Combination therapy may provide a brighter future by reducing tumour growth, resistance and metastasis.
Title: Towards personalized locoregional treatment; assessing the risk of locoregional breast cancer recurrence using the 70-gene signature – an analysis from the EORTC 10041/BIG 03-04 MINDACT trial

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Body: Purpose: The locoregional recurrence rate (LRR) of breast cancer (BC) has decreased substantially over the last three decades. The 70-gene signature (70-GS) has been demonstrated to improve prediction of the risk of distant metastases in BC and select those patients in whom adjuvant chemotherapy may be safely omitted. We aimed to evaluate whether the 70-GS can discriminate between patients with a low or high risk of LRR to allow personalized recommendations for post-operative radiotherapy.

Patients and Methods: All patients enrolled in the EORTC 10041/BIG 03-04 MINDACT trial with a known clinical (C) risk, per a modified version of Adjuvant!Online, and genomic (G) risk assessment, per 70-GS, were categorised for type of surgery: breast-conserving surgery (BCS) or mastectomy. Time to LRR was calculated as the time to first event (local or regional whichever occurred first). Distant metastasis and death were considered as competing risks. The 5-year LRR and 95%CI was calculated for type of surgery both overall and for the different risk groups (C-low/G-low, C-low/-high, C-high/G-low and C-high/G-high). The statistical analysis plan for this project was prospectively defined.

Results: Among the 6693 enrolled patients, 5470 (81.7%) underwent BCS and 1223 (18.3%) patients received a mastectomy. Breast or chest wall RT was administered to 98% of patients who underwent BCS and 29% after mastectomy. 155 patients experienced a LRR as first event (120 and 35 patients in the BCS and mastectomy groups, respectively). Cumulative incidence of LRR was similar by nodal status.

In the 5470 patients treated with BCS, 88 patients had a (73.3%) local relapse and 49 (40.8%) regional relapse, meaning 17 patients that underwent BCS had (14.2%) concomitant local and regional relapses. In the BCS group, 3578 (65.4%) patients were G-low and 1891 (34.6%) were G-high. The 5-year LRR rate was 2.13% (95% CI 1.72-2.55) overall and 1.39% (95% CI 0.97-1.80) in the G-low versus 3.56% (95% CI 2.66-4.46) in the G-high group.

In patients treated with mastectomy, 716 (58.5%) patients were G-low and 507 (41.5%) were G-high. The 5-year LRR rate was 2.47% (95% CI 1.56-3.37) overall, 0.73% (95% CI 0.09-1.37) in patients at G-low risk and 4.93% (95% CI 2.95-6.92) in patients at G-high risk.

Conclusion: In this study we observed a lower 5-year locoregional recurrence rate in patients with a low risk 70-GS profile as compared to those with a high genomic risk profile, both in the BCS group as well as after mastectomy. Multivariable analyses will be performed and presented, to evaluate the independent prognostic value of the 70-GS with respect to LRR. This information could be used to tailor radiotherapy according to expected benefit.
Title: Differential benefit of docetaxel-based chemotherapy in breast cancer patients according to baseline body mass index

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Body: Background:
According to the latest estimates, 63% of the women in the US are either overweight or obese. Lipophilic drugs, such as docetaxel (T), have a high affinity for adipose tissue and a resulting higher volume of distribution (Vd). We hypothesized that the local and distant efficacy of T-based chemotherapy would differ according to the patient's adiposity, as estimated by the body mass index (BMI).

Patients and methods:
We retrospectively analyzed data from all the patients from a prospective neo-adjuvant trial comparing fluorouracil, epirubicin and cyclophosphamide (FEC) to T-ET (EORTC10994, NCT00017095, n=1,856) and from an adjuvant trial comparing two non-T regimens to two T-containing regimens (BIG 2-98, NCT00174655, n=2,887). No capping for patients with a body surface area >2.0m² was recommended in the study protocols, except after the 1st amendment of BIG 2-98 when already 75% of the patients were accrued. Three subgroups of BMI were considered: BMI<25 (lean, L), 25 ≤ BMI<30 (overweight, Ov) and BMI ≥ 30 (obese, Ob). Pathological complete response (pCR) was defined, as in the initial study, as pT0/is. Distant metastasis free (DMFS) and overall survival (OS) were considered as endpoints.

Results:
In both trials, the distribution of the BMI categories and the associations of BMI with clinico-pathological parameters were similar in the T and non-T groups.
In the neo-adjuvant trial, there was no difference in pCR rate between the two treatment arms considering all patients (FEC: 24%, T-ET: 27%), however a significant decrease in pCR rate was observed with increasing BMI in the FEC arm (L: 26%, Ov: 23%, Ob:17%, p=0.049), but not in the T-ET arm (L: 27%, Ov: 26%, Ob:27%, p=0.802). These pCR rates were not explained by differences in relative dose intensity (RDI). Exploratory analyses revealed that obese patients with ER-positive cancer that achieved pCR in the T-ET arm presented with a worse DMFS and OS.
In the adjuvant trial, there was no difference in DMFS and OS according to BMI in the non-T group, while a decreased DMFS and OS was observed with increasing BMI category in the T-group, which could not be explained by RDI. Subgroup analyses within the BMI categories demonstrated that the differential efficacy of T was limited to the L patients (HRadj OS T vs non-T=0.76 (0.60-0.96), p=0.02).

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<tr>
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<th>DMFS</th>
<th>OS</th>
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<tr>
<td>Non-T group</td>
<td></td>
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<tr>
<td>Ov vs L</td>
<td>1.19 (0.90-1.58), 0.21</td>
<td>0.96 (0.72-1.28), 0.80</td>
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<tr>
<td>Ob vs L</td>
<td>1.09 (0.77-1.55), 0.62</td>
<td>1.09 (0.78-1.52), 0.61</td>
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<tr>
<td>T group</td>
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<tr>
<td>Ov vs L</td>
<td>1.22 (0.99-1.57), 0.06</td>
<td>1.24 (1.00-1.54), 0.05</td>
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<tr>
<td>Ob vs L</td>
<td>1.52 (1.20-1.92), &lt;0.001</td>
<td>1.68 (1.32-2.13), &lt;0.001</td>
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Conclusion:
This analysis of two large trials highlights for the first time a differential response to T according to BMI at the local and distant level. We hypothesize that due to its lipophilic nature, T is characterized by a high affinity for adipose-rich tissues such as the
mammary gland and provides a stable pCR rate across the BMI categories. However, due to its higher Vd, it is associated with a decreased efficacy at the distant level in patients with increased BMI. The differential efficacy of T compared to non-T chemotherapy regimens according to BMI calls for a body composition-based re-evaluation of the risk-benefit ratio of T-use in breast cancer.
Title: Efficacy and tolerability of tremelimumab in metastatic triple-negative breast cancer

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Body: Background: The clinical activity and safety profile of anti-CTLA-4 antibody monotherapy in metastatic triple-negative breast cancer (TNBC) are not yet known. Tremelimumab, a selective human IgG2 monoclonal antibody targeting CTLA-4, was evaluated in patients with advanced solid tumors in a Phase 2, multicenter, open-label study (NCT02527434). We report a planned analysis of safety and efficacy in a cohort of patients with metastatic TNBC.

Methods: The TNBC cohort included adult patients with histologically or cytologically confirmed metastatic TNBC who had progressed on, could not tolerate, were ineligible for, or refused prior chemotherapy. Patients received tremelimumab 750 mg IV every 4 weeks (Q4W) for 7 doses, followed by 750 mg Q12W for 2 doses, for a total of 12 months or until progression, initiation of another anticancer therapy, or unacceptable toxicity. The primary endpoints were safety (evaluated by Common Terminology Criteria for Adverse Events [CTCAE] v4.0) and confirmed objective response rate (ORR).

Results: As of April 5, 2017, 12 patients with metastatic TNBC from 4 sites in 3 countries had received treatment (as-treated population). All were eligible for the efficacy analysis. All had Stage 4 disease at study entry and had received prior anticancer therapy (1 to 4 prior lines). Median follow-up for overall survival was 7.6 months (range, 0.8–12.0). Objective response was observed in 1 patient, who achieved a partial response (ORR 8.3%; 95% CI, 0.2, 38.5). This response was observed at the first scheduled scan (time to response, 3.5 months) and lasted 11 months. No patient had a complete response or stable disease. Median progression-free survival was 3.6 months (95% CI, 0.8, 4.0) and median overall survival was not estimable. Six patients (50.0%) experienced adverse events (AEs) related to tremelimumab, but there were no grade 3/4 treatment-related AEs. All-causality AEs led to discontinuation in 2 patients (16.7%). There were no treatment-related deaths. Biomarker analysis will be described in the presentation.

Conclusion: Tremelimumab 750 mg Q4W demonstrates favorable clinical activity and an encouraging and manageable safety profile in patients with metastatic TNBC.
Title: Use of seleno-immunoconjugates for triple negative breast cancer treatment

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Body: Problem: Triple Negative Breast Cancer accounts for 15-20% of breast cancers. The lack of therapeutic targets (estrogen receptor, progesterone receptor and HER2) correlates with a more aggressive phenotype and consequently, harsher treatment strategies. Furthermore, overexpression of Vascular Endothelial Growth Factor (VEGF), facilitating angiogenesis, is also associated with TNBC. Avastin, a humanized monoclonal antibody against VEGF, can be used to target TNBC cells with clinically relevant expression. However, anti-estrogen and antibody-based treatments that target the ER and HER2 respectively, (i.e., tamoxifen and Herceptin) in other breast cancer histotypes are not applied in TNBC treatments due to lack of efficacy. Thus, to date, targeted treatments with decreased side effects has not been a viable option.

Purpose: It is known that ER- and HER2- does not mean the absence of receptors, but rather, expression levels so low as to make clinical targeting strategies ineffective. Upon endocytosis, selenides generate superoxide free radicals within cells via redox cycling of selenium intermediates. Superoxide generation by selenium is reported to cause cellular apoptosis due to increased oxidative stress. Combining this information, we hypothesized that selenium conjugated to current, clinically-relevant, targeted therapies for breast cancer would increase their efficacy significantly, making them potentially viable as treatment options for TNBC. Furthermore, due to the differences in redox cycling, we further hypothesized that the toxic effects of selenium would be attenuated in normal mammary epithelial cells (HMEC).

Methods: In the present study, selenium was covalently attached to Herceptin and Avastin to target HER2 receptors and VEGF in the TNBC cell lines MDA-MB-231 and MDA-MB-468, respectively. Selenium (Se) attachment to monoclonal antibodies was measured by ICP-MS. MDA-MB-231, MDA-MB-468 cell lines and HMEC were treated with Sodium Selenite (2 µg, 5 µg and 10 µg as Se); Herceptin (26.22 µg, 65.55 µg and 131.3 µg); Se-Herceptin (2 µg/26.22 µg; 5 µg/65.55 µg; 10 µg/131.3 µg); Avastin (30.75 µg, 76.76 µg, 153.76 µg) and Se-Avastin (2 µg/30.75 µg; 5 µg/76.75 µg; 10 µg/153.75) and assayed at 24, 48, 72, 96, 120 and 144 hours. The rate of cell proliferation (cell counts), metabolism (MTT), superoxide formation (DHE), cytotoxicity (Trypan Blue) were investigated.

Results: Selenium labeled immunoonjugates demonstrated Selenium concentration and time dependent cytotoxicity in both TNBC cells tested. Between 24-144 hours of treatment, Selenium immunoconjugates showed significant decreased cell proliferation. Moreover, HMEC demonstrated significantly lower cytotoxicity and maintained high (>90%) viability during treatment. Selenium conjugated mAbs generated significant intracellular superoxide proving to be effective targeted therapy against TNBC.
Title: Efficacy of sacituzumab govitecan (IMMU-132), an anti-Trop-2-SN-38 antibody-drug conjugate, as $\geq 3$rd-line therapeutic option for patients with relapsed/refractory metastatic triple-negative breast cancer (mTNBC)

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Body: Background: Metastatic TNBC has an aggressive course with limited therapy options and poor survival. Sacituzumab govitecan (IMMU-132) is a novel antibody drug conjugate consisting of SN-38, the active metabolite of the topoisomerase I inhibitor, irinotecan, conjugated to a humanized mAb targeting Trop-2, which is highly expressed in most epithelial cancers, including TNBC. A phase I/II basket trial (NCT01631552) was conducted in patients (pts) with multiple, advanced epithelial cancers. We previously reported results in mTNBC based on enrollment of 69 pts between July 2013 and March 2016 who had received a median of 5 prior therapies since initial diagnosis (objective response rate \textbf{ORR} = 30\%, median duration of response \textbf{DOR} = 8.9 months; Bardia et al., JCO, 2017). In 2016, Sacituzumab govitecan was granted Breakthrough Designation based on this encouraging data, and we pursued further enrollment of patients with TNBC who had received at least 2 prior therapies for their metastatic disease.

Methods: Pts received sacituzumab govitecan on days 1 and 8 of a 21-day cycle until progression or unacceptable toxicity. Eligibility included patients with mTNBC, at least 2 prior lines of therapy for metastatic disease, and measurable disease by CT or MRI. Efficacy was assessed locally by RECIST 1.1 and confirmed by an independent centralized blinded review. ORR, DOR, progression-free survival (PFS) and overall survival (OS) were determined for all pts. Adverse events (CTCAE v4.0), pharmacokinetics, immunogenicity, and evaluation of Trop-2 expression levels on archived tumor samples were also evaluated.

Expected Results: To date, over 100 patients with mTNBC previously treated with $\geq$2 prior regimens in the metastatic setting have been accrued with the last patient enrolled in Feb 2017, and final results are currently being analyzed with plans for regulatory submission based on this data. Complete efficacy results, including ORR, DOR, PFS, OS together with safety data, pharmacokinetics, immunogenicity, and tumor Trop-2 expression results will be provided.

Expected Conclusions: The final conclusions will be based on efficacy and safety profiles of sacituzumab govitecan in patients with relapsed/refractory mTNBC previously treated with at least 2 prior regimens in the metastatic setting. Updated data will be presented.
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**Body: Background:** Mutations in the PIK3CA gene represent a source of heterogeneity in HER2-positive breast cancer, with potential impact on prognosis and treatment sensitivity. This study explores the frequency, association with clinicopathological features, prognostic and predictive impact of PIK3CA mutations in the phase III, randomized, multicentric, non-profit adjuvant Short-HER trial.

**Methods:** The Short-HER trial randomized 1254 patients with HER2-positive early breast cancer to receive 9 weeks or 1 year of adjuvant trastuzumab combined with chemotherapy. Non-inferiority of the short arm was not demonstrated with the frequentist approach, but the Bayesian method based on the observed data estimated that the short arm has a 78% probability of not being inferior to the long treatment. Tumor tissue samples were centralized for 904 patients (72%). At present, genomic DNA has been extracted from n=776 formalin-fixed paraffin-embedded samples with a tumor cell content of ≥18%. PIK3CA hot-spot mutations in exon 9 (E542K, E545K-A-G, Q546E-K) and exon 20 (M1043I, H1047R-L-Y, G1049R-S) were analysed by using Pyrosequencing method.

**Results:** A mutation of the PIK3CA gene was detected in 21.8% of the 776 genotyped patients (n=169 mutated; n=607 wild type). Mutations in exon 9 and 20 occurred in 76 (9.8%) and 93 (12%) cases, respectively. A significant association with hormone receptor status was observed, with PIK3CA mutation occurring in n=131 (23.6%) of 554 hormone receptor-positive patients and in n=38 (17.1%) of the 222 hormone receptor-negative cases (p=0.046). No significant association with stage and nodal status was observed. At present, a disease-free survival (DFS) event has been reported in 10.7% of mutated patients and in 15.2% of wild-type patients. DFS rates at 3 and 5 years in the mutated and wild type groups were as follows: 95.8% (95% CI 91.4-98.0) in mutated vs 92.5% (95% CI 90.1-94.4) in wild type at 3 years; 91.1% (95% CI 85.5-94.7) in mutated vs 85.9% (95% CI 82.7-88.5) in wild type at 5 years. The DFS cox regression model did not show a statistically significant difference: HR 0.69 (90% CI 0.45-1.06). Exploratory DFS analyses comparing the short vs long treatment arm were conducted separately in the mutated and wild-type groups. The HR was 0.92 (90% CI 0.42-2.00) in the mutated group and 1.12 (90% CI 0.79-1.58) in the wild type group.

**Conclusions**

PIK3CA mutated patients had numerically higher DFS rates at 3 and 5 years compared to wild-type patients, but the difference was not statistically significant at this time. Follow up is ongoing, updated analyses will be presented at the meeting. Supported by AIFA (FARMS5KR) and AIRC (MFAG-15938).
Title: Trastuzumab versus observation for high-risk, non-metastatic, HER2 non-amplified breast cancer with circulating tumor cells (EORTC 90091-10093 treat CTC): A European, multicenter, randomized phase 2 trial

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Body: Background: It is known that trastuzumab improves the outcome of women with HER2 positive breast cancer. We aimed to assess whether trastuzumab decreases the detection rate of Circulating Tumor Cells (CTCs) compared to observation in women with HER2 non-amplified, non-metastatic breast cancer.

Methods: The EORTC 90091-10093 Treat CTC is a randomized phase 2 study, conducted in 70 hospitals in 5 European countries. Patients with centrally confirmed HER2 non-amplified breast cancer and at least 1 CTC per 15mL of blood by CellSearch® following surgery and (neo)adjuvant chemotherapy (CTC test at baseline) were randomized (1 :1) to 6 cycles of trastuzumab intravenously (1 cycle at loading dose of 8mg/Kg followed by 5 cycles at 6mg/kg) versus observation. Randomization was stratified for center, locally confirmed estrogen receptor (ER) status and adjuvant versus neoadjuvant chemotherapy. The primary endpoint was rate of detection of at least 1 CTC per 15mL of blood by 6 cycles of trastuzumab or 18 weeks of observation (CTC test at week 18). Secondary endpoints were invasive disease-free survival (iDFS) and cardiac safety.

Findings: Between April 30 2013 and October 17 2016, 1317 patients were screened, 95 (7.2%) had detectable CTC(s) and 63 (4.8%) were randomly assigned to trastuzumab (n=31) or observation (n=32). Fifty-eight patients were evaluable for the primary endpoint of CTC detection at week 18; 29 in each arm. In 9 out of the 58 patients, CTC(s) were still detected in their blood sample at week 18; 5 in the trastuzumab arm and 4 in the observation arm (one-sided Fisher exact test, p = 0.765). Further accrual to the study was stopped for futility for the primary endpoint by an Independent Data Monitoring Committee. Median follow-up for the outcome data at the time of database lock was 13 months (IQR 4-16.5). The 1-year iDFS was 93.8% (95% CI 77.3-98.4) in the observational arm versus 84.8% (95% CI 63.4-94.2) in the trastuzumab arm. No grade 2-4 cardiac events were observed in the trastuzumab arm.

Interpretation: Trastuzumab does not decrease the detection rate of Circulating Tumor Cells in HER2 non-amplified, non-metastatic breast cancer.
Body: Background
In a prior proof-of-principle single centre study we demonstrated that detection of circulating tumor DNA (ctDNA) in the adjuvant setting, after completion of chemotherapy and surgery for early stage breast cancer, was associated with a high risk of early relapse. We present an independent multi-centre validation study to assess the clinical validity of ctDNA analysis in anticipating disease relapse.

Methods
A cohort of 168 women presenting with early stage breast cancer were prospectively recruited into two multi-centre sample collection studies (ChemoNEAR and Plasma DNA). All patients were scheduled to received chemotherapy and surgery. Primary tumours were sequenced using an amplicon-based gene panel to identify driver somatic mutations, in a single central laboratory. Personalised digital PCR assays were developed for each individual patient, and validated with controls including clonal haematopoesis screening. Mutations were tracked in plasma taken at baseline, at a single post-treatment time point (2-6 weeks after completion of surgery and chemotherapy) and with serial plasma samples taken every 6 months in the adjuvant setting.

Results
The independent validation series has a median follow-up of 23.7 months. Circulating tumour DNA analysis is ongoing, and will be completed by the late breaking abstract deadline. The primary objective will be to assess the association between detection of ctDNA post-treatment and disease free survival (excluding contralateral primaries), along with lead time between ctDNA detection and disease relapse.

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Title: Clinical significance of somatic genomic mutations in triple negative breast cancer survivors in complete clinical remission

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Body: Background: Triple negative breast cancer (TNBC) remains the most aggressive subtype of breast cancer in which up to 1/3 of all patients will relapse early and distantly. This prospective study examined the utility of serial liquid biopsies using a multi-template approach (identification and analysis of cell-free DNA (cfDNA) and circulating tumor cells (CTCs)) to monitor and predict for recurrence.

Methods: 211 patients (average 53 yrs.) with confirmed diagnosis of TNBC, within 3 years of completion of therapy (mean 25 mos.), and in full remission were enrolled. Liquid biopsies were performed quarterly to look for cancer specific genomic mutations (>1.0% frequency) not seen in germ line controls, but in cfDNA and/or CTCs using a custom 27-gene breast cancer panel. Prior validation of our gene panel demonstrated a false positive rate of 0.001-0.0007% in normal cfDNA and cell-based controls.

Results: 850 samples (average 4.0 per patient) have been collected to date. 22.8% of samples were found to bear mutations in cfDNA alone, 19.8% in CTCs alone, and 4.28% of both cfDNA and CTCs. 163 patients (77.25%) were found to have mutations in at least one sample collected. Of patients bearing mutations, 11 (6.7%) developed documented evidence of recurrence, one in the axilla and the others with visceral/distant metastases. Of patients who have recurred, all displayed persistent presence of mutations on consecutive blood draws. Conclusions: 93.3% of patients found to have mutations on at least one draw did not have presence of a mutation on successive draws and have remained clinically NED. In patients who recurred, all displayed persistent evidence of mutations on serial draws. When the same mutation was seen on successive draws, the risk of recurrence was statistically significant. This study highlights the need for serial monitoring using a multi-template approach to avoid over treatment of patients who do not develop clinical recurrence despite having mutations in blood.
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Body: The degree of intrinsic and interpatient phenotypic heterogeneity and its role in tumour evolution is not yet understood. Phenotypic divergence can be achieved via the inheritance of alternative transcriptional programs. Cell-type specific transcription is maintained through the activation of epigenetically-defined regulatory regions including promoters and enhancers. In this work, we have annotated the epigenome of 47 oestrogen-receptor (ERa)‐positive breast cancer specimens and developed strategies to extrapolate phenotypic heterogeneity from the regulatory landscape, identifying key regulatory drivers commonly shared across patients. Regulatory drivers are highly enriched for the transcription factor YY1. YY1 engages all estrogen-inducible enhancers in ERa breast cancer cells and defines a critical subset of functional ERa binding sites. YY1 is also essential for maintaining the expression of ERa target genes that mediate resistance to endocrine treatment. We further show that H3K27ac levels at active enhancer elements can be used as a qualitative surrogate to estimate intra-clonal tumor heterogeneity, and to track expansion and contraction of subpopulations throughout breast cancer progression. Tracking YY1 and SLC9A3R1 phenotypic clones by epigenetic mapping and immunocytochemistry shows that metastatic tumors are dominated by phenotypic clones originally underrepresented at diagnosis that expand under endocrine treatments. Collectively, our data demonstrate the active role of therapeutic treatments as a dominant factor underlying epigenetically driven phenotypic evolution in ERa breast cancer in vivo.
Title: Is there unknown BRCAness in the breast cancer

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Body: BRCAness, or Homologous recombination deficiency (HRD), is a tumor phenotype associated with the inactivation of BRCA1 or BRCA2, two major genes from Homologous recombination pathway. Genomic signatures of BRCAness define a genomic phenotype characterized by an increased rate of the large-scale chromosomal alterations. Breast tumors positive for genomic BRCAness (HRD) are mostly explained by the inactivating mutations (germline or somatic) in BRCA1 or BRCA2 or by the methylation of BRCA1 promoter. Beside BRCA1/2 only RAD51C inactivation (mostly by the promoter methylation) was shown to be unambiguously associated with the genomic BRCAness [1]. The question is how many breast tumors display BRCAness that is not explained by BRCA1/2 or RAD51C inactivation. Using the LST (Large-scale state transitions) genomic signature we estimated unknown BRCAness to be ~10% (6/72) in the Triple Negative (TNBC) and ~20% (11/56) in luminal and HER2+ subtypes of the in-house (Institute Curie) well-characterized cohort of 560 cases [2]. The TCGA breast cancer cohort (BRCA, 941 cases) yielded ~20% (17/86) TNBC and ~30% (19/56) luminal and HER2+ tumors with unexplained BRCAness [2, 3]. These estimations depend on the LST genomic signature, which could produce false positives overestimating unknown BRCAness. The recent study, which is based on the whole genome sequencing (WGS), provided the data and a very accurate method for BRCAness detection (HRDetect) [4]. However, testing for BRCA1 and RAD51C promoter methylation was not exhaustive in this study and this affected estimation of unexplained BRCAness. Aiming at a conservative estimation, we filled the gap in methylation data using gene expression. In order to get a complete view we considered only tumors with available RNA-seq expression data (260 cases). We showed that the methylated cases could be efficiently selected based on the proliferation score (first principal component of E2F target genes) and expression level of BRCA1 and RAD51C genes. We validated the procedure using the TCGA and in-house expression and methylation data. After refining the methylation status of RAD51C and BRCA1 genes we conclude that unknown BRCAness could be estimated as 12% (5/41) in TNBC and 17% (3/17) in luminal and HER2+ subtypes. These results are in a good correspondence with previous data and could be considered as a reasonable estimate for unknown BRCAness (unexplained HRD) in breast cancer. As it was indicated in several studies there is no other gene candidate displaying consistent genomic BRCAness upon inactivation in breast cancers [2, 4].

References
2017 San Antonio Breast Cancer Symposium

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Title: Assessment of breast conserving surgery where the surgery site is marked with blue dye and jelly injection as a golden standard procedure in Japan


Body: Background: For breast conserving surgery (BCS), preoperative localization of the cancer lesion including extended ductal carcinoma in situ is very important so that the safety margin is kept in both palpable and nonpalpable breast cancers. Currently, palpation-guided localization for palpable lesions and wire-guided localization (WGL) or radioguided occult lesion localization (ROLL) for nonpalpable lesions are reported as standard procedures. However, the situation is quite different in Japan, where the indication of BCS is decided based on all results of palpation, mammography, ultrasound and magnetic resonance imaging in most cases. For a lesion, both palpable and nonpalpable, that can be detected by ultrasound, we have performed BCS with blue jelly localization (BJL). This is the most widely used procedure in Japan; however, the appropriateness of this procedure has not been studied. Therefore, we conducted a retrospective study to assess the BJL method.

Methods: The subjects were breast cancer patients who underwent BCS with BJL at Aichi Cancer Center Hospital from 2011 to 2015. Patients who underwent neoadjuvant systemic therapy were excluded. The BJL method is performed as follows. First, the location of the breast cancer is confirmed by ultrasound in the surgical position. Second, we design a resection line with a margin of 1.5-2cm around the lesion and mark it on the skin with a pen. Third, after general anesthesia and sterilization, blue dye (indocyanine green or patent blue) mixed with sterile lubricating jelly (1.5ml:1.5ml) is injected subcutaneously and in the mammary gland at several points along the designed resection line. Last, after skin excision and making a skin flap, we cut along the resection line according to the injected blue dye and palpation. The deep margin is always the fascia of pectoralis major. The margin status was evaluated at 5mm resection margin in our institution; therefore, a clear margin and positive margin were defined as over or less than 5mm from the cut surface, respectively.

Results: 752 lesions in 735 patients were assessed. The patients' median age was 54.0 years, median BMI was 22.0, and median specimen weight was 53.0 g. Among 752 lesions, 242 lesions were nonpalpable and 510 were palpable lesions. Clear resection margins were achieved in 84.7%(205/242) of nonpalpable lesions and in 78.2%(399/510) of palpable lesions. Among the 148 margin-positive cases, 48 cases (32.4%) were positive for invasive cancer and 100 (67.7%) were positive for ductal carcinoma in situ. There was no case of failure of resection of the breast cancer lesion. Re-excision or mastectomy due to positive margin was needed in 11 patients (4.5%) with nonpalpable lesions and in 26 patients (5.1%) with palpable lesions. Among the 37 patients who underwent re-operation, there was no pathological remaining tumor in 27 cases. There were no adverse events such as allergic reaction caused by BJL.

Conclusion: BJL is a convenient and safe method. Although the limitations of this study are its retrospective nature and single institution analysis, the clear margin rate for nonpalpable lesions did not differ from those of WGL or ROLL. BJL is recommended as a reliable method in BCS.
2017 San Antonio Breast Cancer Symposium

Title: Can we omit sentinel lymph-node biopsy in the patients with negative conversion after neoadjuvant chemotherapy in initially locally advanced breast cancer with positive node?

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Body: Background: Quality of life and long-term effects of treatment have become increasingly important for breast cancer patients due to improved survival outcomes. Sentinel lymph node biopsy (SLNB) has been investigated after neoadjuvant chemotherapy (NAC) in patients with locally advanced breast cancer. Many associated clinical trials are in progress like SENTINA or ACOSOG-Z1071 but it has shown mixed results. The aim of this study was to determine the feasibility of sentinel lymph node biopsy replacing axillary lymph node dissection (ALND). Materials and methods: We identified consecutively 447 women with invasive breast cancer who underwent surgical resection after NAC at our institution between April 2004 to August 2013. Clinico-pathological data obtained from prospective collecting medical database were analyzed retrospectively. Among them, only 359 patients had evidences of axillary, internal mammary of supraclavicular lymph node metastasis at the time of diagnosis with 123 cytology-proven patients and 236 imaging-guided patients. We investigated a conversion rate from node positive to negative after NAC. We compared the loco-regional recurrence rate (LRR) according to the lymph node conversion and the axillary surgery type (sentinel biopsy only or complete axillary dissection). Results: We started SLNB only in patients who underwent NAC with node positive breast cancer from March 2005. Because of no credible evidences before that time, complete axillary dissection was done after SLNB. SLNB was successfully performed in 156 (46.3%) patients. Tumor nonresponse and extensive residual nodal disease were found to be associated with detection failure of sentinel nodes. In 115 (32.0%) patients, nodal status converted from positive to negative. Local recurrence was 7 (6.1%) cases in node conversion to negative patients and 12 (4.9%) cases in non-conversion patients, and it showed significant difference (p=0.002). In node conversion to negative patients, the LRR was 5.0% in SLNB only group and 6.7% in ALND group. In non-conversion patients, the LRR was 5.6% in SLNB only group and 4.8% in ALND group. Regardless of axillary surgery type, LRR had no statistically difference between each groups according to node conversion status (p=0.780(node negative) and p=0.945(non-conversion)). The number of patients treated with radiotherapy (RTx.) was significantly different between the node conversion to negative group and non-conversion group (p=0.015); node conversion group were more likely to have radiotherapy (112 (97.4%)) than the patients in the non-conversion group (220 (90.2%)). Lymphedema was observed statistical differently in 17.1% of SLNBx. group but in 47.0% of ALND group (p<0.05). Limited range of motion showed no difference, it was observed in 5 patients (6.6%) of SLNBx. group and in 13 patients (4.6%) of ALND group (p=0.481). Conclusions: In our study, there was no significant difference in LRR as above. The complication rate was significantly lower in the SLNBx. groups regardless of node conversion after NAC. This results can be an answer to the question about SLNB can omit in patients with conversion to node negative after NAC in initially node positive breast cancer.
**2017 San Antonio Breast Cancer Symposium**

Publication Number: P3-06-11

**Title:** Synergies between comedications, immune infiltration and response to treatment: exploratory analysis of a cohort of breast cancer patients treated by neoadjuvant chemotherapy

Anne-Sophie Hamy\(^1,2\), Constance Val de Lièvre\(^3\), Enora Laas\(^3\), Lauren Darriques\(^3\), Maël Priour\(^4\), Julien Guerin\(^4\), Thomas Balezeau\(^4\), Alain Livartowski\(^4\), Jean-Yves Pierga\(^5\), Laurence Escalup\(^6\), Bernard Asselain\(^1\), Roman Rouzier\(^3\), Marick Lae\(^7\), Diane Decroze\(^7\), Alice Pinheiro\(^1,2\), Cecile Laurent\(^1,2\) and Fabien Reyal\(^1,2,3\).


**Body:** Purpose: Epidemiologic studies showed a protective effect of several medications classes either on breast cancer incidence or recurrence. Additionally, chronically used medications for concomitant illnesses may modify chemotherapy pharmacokinetics/dynamics. The aim of the study is to evaluate the association between comedications taken during neoadjuvant chemotherapy (NAC) and pathologic complete response (pCR) rates.

**Methods:** We identified 1016 patients with primary breast cancer (BC) receiving NAC between 2002 and 2011. We analyzed the association between chronic comedications and pathological complete response (pCR) in the whole population (WP) and in the three BC subtypes (luminal, HER2-positive, triple negative (TNBC)). Tumor infiltrating lymphocytes (TIL) levels were available on pretreatment biopsies for 559 patients (55%).

**Results:** 502 patients (49.4%) declared using a chronic comedication. The main three pharmacotherapeutical classes represented were cardiology (n=343, 25.3%), psychiatry (n=327, 24.1%), and endocrinology (n=191, 14.1%). The use of any comedication was independently associated with higher pCR rates than when no comedication (26.7% versus 19.8%, OR=1.47 95%CI [1.04-2.08], \(p=0.028\)). We retrieved significant associations between pCR and use of several pharmacological classes known to be protective for breast cancer: biguanids (yes versus no: pCR: 40.9% vs 22.8%, \(p=0.054\) [WP]), angiotensin II receptor antagonists (yes versus no: pCR: 70.6% versus 34.5%, \(p=0.006\) [HER2-positive BC]), and corticoids (yes versus no: pCR: 33.3% vs 5.4%, \(p=0.003\) [luminal BC]). We also identified unexpected associations: hypnotics (yes versus no: pCR: 35.3% vs 22.6%, \(p=0.039\); anxiolytics (yes versus no: pCR 57.5% vs 34.2%, \(p=0.005\) [TNBC]) and proton pump inhibitors (PPI, yes versus no: pCR: 59.1% vs 35.5%, \(p=0.03\) [TNBC]). Some classes were associated with both higher pCR rates and higher TILs levels: hypnotics (TILs: 33.1% vs 22.9%, \(p<0.001\) [WP]; biguanids (TILs: 34.3% vs 23.2%, \(p=0.02\) [WP]; PPI: (37.5% vs 26.6%, \(p=0.03\) [TNBC]).

<table>
<thead>
<tr>
<th>Pharmacologic Subclass and BC subtype</th>
<th>% Pathological complete response</th>
<th>OR</th>
<th>95% CI</th>
<th>(p)</th>
<th>mean TILs levels</th>
<th>(p)</th>
</tr>
</thead>
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<tr>
<td><strong>Whole population</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Thyroid hormones</td>
<td>No</td>
<td>22.7</td>
<td>1</td>
<td></td>
<td>22.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>31.7</td>
<td>1.59 [0.9-2.72]</td>
<td>0.10</td>
<td>31.7</td>
<td>0.01</td>
</tr>
<tr>
<td>Biguanids</td>
<td>No</td>
<td>22.8</td>
<td>1</td>
<td></td>
<td>23.18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>40.9</td>
<td>2.34 [0.95-5.49]</td>
<td>0.05</td>
<td>34.3</td>
<td>0.02</td>
</tr>
<tr>
<td>Anxiolytics</td>
<td>No</td>
<td>22.2</td>
<td>1</td>
<td></td>
<td>23.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>30.1</td>
<td>1.52 [1.01-2.25]</td>
<td>0.04</td>
<td>22.6</td>
<td>0.62</td>
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<tr>
<td>Hypnotics</td>
<td>No</td>
<td>22.6</td>
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<td></td>
<td>22.9</td>
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<td>Yes</td>
<td>35.3</td>
<td>1.87 [1.01-3.35]</td>
<td>0.04</td>
<td>33.1</td>
<td>&lt;0.001</td>
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<td><strong>TNBC</strong></td>
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<td></td>
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<tr>
<td>Proton pump inhibitors</td>
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<tr>
<td></td>
<td>Yes</td>
<td>59.1</td>
<td>2.63 [1.1-6.56]</td>
<td>0.03</td>
<td>37.5</td>
<td>0.03</td>
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<tr>
<td>Anxiolytics</td>
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<td></td>
<td>27</td>
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<tr>
<td>Drug/Condition</td>
<td>Yes</td>
<td>57.5</td>
<td>2.61</td>
<td>[1.33-5.18]</td>
<td>0.005</td>
<td>31.3</td>
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</tr>
<tr>
<td>Hypnotics</td>
<td>No</td>
<td>36.1</td>
<td>1</td>
<td></td>
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<tr>
<td></td>
<td>Yes</td>
<td>52.6</td>
<td>1.97</td>
<td>[0.77-5.09]</td>
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<td><strong>HER2-positive</strong></td>
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<tr>
<td>Angiotensin II antagonists</td>
<td>No</td>
<td>34.5</td>
<td>1</td>
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<tr>
<td></td>
<td>Yes</td>
<td>70.6</td>
<td>4.56</td>
<td>[1.63-14.75]</td>
<td>0.006</td>
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<tr>
<td>Lipid modifying agents</td>
<td>No</td>
<td>35</td>
<td>1</td>
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<tr>
<td></td>
<td>Yes</td>
<td>75</td>
<td>5.56</td>
<td>[1.61-25.58]</td>
<td>0.01</td>
<td>28.3</td>
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<tr>
<td><strong>Luminal</strong></td>
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<td></td>
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<td></td>
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<tr>
<td>Corticosteroids</td>
<td>No</td>
<td>5.4</td>
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<tr>
<td></td>
<td>Yes</td>
<td>33.3</td>
<td>8.73</td>
<td>[1.76-35.3]</td>
<td>0.003</td>
<td>13</td>
</tr>
</tbody>
</table>

**Conclusion:** The neoadjuvant setting is an optimal framework to both validate and identify associations between comediations and response to treatment. Large-scale validation studies are needed to refine which non-cancerous drugs should be repurposed as enhancers of anticancer treatment.
2017 San Antonio Breast Cancer Symposium

Publication Number: P3-07-11

Title: Potent CIB1-based combination therapies in triple-negative breast cancer

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Body: Background: Triple-negative breast cancer (TNBC) patients are limited to standard chemotherapy and surgery, and the overall efficacy of these treatments has been marginal due to toxicity and recurrence. Therefore, there is a pressing need for new targeted and combination therapies that are safe and durable. Our lab previously identified CIB1 as a potentially safe and effective target in TNBC. The purpose of this study is to test the efficacy of novel CIB1-based combination treatments in selectively inducing tumor cell death and understanding the underlying mechanism(s).

Methods: We targeted CIB1 by RNA interference in MDA-MB-436 TNBC and ME16C normal breast epithelial cells. CIB1 targeting was combined with either docetaxel, a chemotherapeutic agent, or TRAIL ligand, a known tumor-specific targeting agent. Cell death was quantified via an automated trypan blue exclusion assay using flow cytometry. Cell death mechanisms including apoptosis and necroptosis were analyzed by Western blotting.

Results: CIB1 depletion in combination with docetaxel significantly increased cell death and caspase-mediated apoptosis in MDA-MB-436 but not ME16C cells. This combined treatment activated death receptor (caspase-8) but not mitochondrial (caspase-9) apoptotic signaling. Moreover, this combined treatment has the potential to be durable since it induces cell death in docetaxel-resistant MDA-MB-436 cells. Interestingly, the pan-caspase inhibitor (z-VAD) only partially blocked cell death and instead may have cross-activated necroptosis (caspase-independent cell death). We also discovered that the combination of CIB1 depletion and TRAIL ligand was significantly more potent in killing MDA-MB-436 cells while sparing normal cells. This profound increase in TNBC cell death was also associated with increased caspase-8 activation.

Conclusion: Our results suggest that CIB1 targeting combined with docetaxel is both efficacious and well tolerated by normal cells. The increased activity of death receptor (caspase-8)-mediated apoptosis appears to be the predominant mechanism underlying the enhanced TNBC cell death. Additionally, this combined treatment was effective in killing docetaxel-resistant TNBC cells, suggesting a viable option to overcome resistance. Finally, the highly potent combination of CIB1 depletion and TRAIL ligand shows minimal effects on normal cells. Given that TRAIL has been well tolerated in clinical trials although with limited efficacy, this novel therapeutic combination has the potential to provide an effective and promising therapeutic option for TNBC patients.
Purpose: Provide an update of the American Joint Commission for Cancer (AJCC) TNM Staging System in order to incorporate contemporary concepts of biology and commonly used biomarkers into cancer staging.

Background: The AJCC TNM Staging System was first developed in 1959 to provide a prognostic tool based on common histopathologic information determined at the time of definitive surgery. This tool was intended to identify patients in differing prognostic categories, so that physicians would select optimal standard treatment and standardize comparison of treatments across clinical trials. Since 1959, there have been seven editions to integrate evolving knowledge into the Staging System and facilitate its use by practicing physicians. Since the publication of the 7th Edition in 2009, new information about tumor biology and prognosis has appeared indicating a need to update the System.

Methods: The AJCC empaneled a multidisciplinary team of experts to review, revise and update the Breast Chapter of the TNM Staging System. The panel included 20 individuals, with representation from pathology, surgery, medical, and radiation oncology, imaging, tumor registries and representatives from the AJCC. The panel reviewed the literature, especially relevant manuscripts published since the 7th edition, and met by conference call and in person to discuss predetermined issues; some related to clarification of the previous edition based on input from the breast cancer community; others were based on the expanding knowledge and utilization of biomarkers (tumor grade and proliferation rate, estrogen and progesterone receptor expression, HER2) as well as multifactorial prognostic panels, including those based on gene expression profiling. AJCC definitions of levels of evidence and guidelines to harmonize staging systems across the oncology spectrum were followed whenever appropriate.

Results: A number of areas reportedly confusing in the previous edition were clarified. Lobular carcinoma in situ was removed from the Staging System. While preserving a staging System based on tumor size and extent of lymph node involvement, a second and third-tier of prognostic modifiers was introduced to incorporate the prognostic influence of grade, hormone receptor expression, and HER2 amplification/overexpression. In addition, and based on emerging evidence from prospective/retrospective analyses, several genomic prognostic platforms were recommended for identification of highly favorable prognostic groups, independent of their anatomic/histologic classification. Utilizing this new system that integrated tumor size, lymph node involvement, grade, ER, PR and HER2 resulted in greater prognostic precision. In addition, based on this new system, about 40% of the tumors classified based on tumor size and lymph node involvement moved to different stages: some were upstaged, while some others were down-staged.

Conclusion: The 8th Edition of the AJCC TNM Staging System provides a more precise but flexible platform for prognostic classification based on histopathology alone or the same modified by commonly used biomarkers and/or multifactorial prognostic panels, in keeping with evolving knowledge of the biology of breast cancer.
Title: Impact of long-term cholesterol-lowering therapy on clinical outcomes in women with breast cancer

Yun R Li¹,², Laura Steel¹, Elena Carrigan¹, Jenny Nguyen¹, Austin Williams¹, Alycia So¹, Ronac Mamtani³ and Julia C Tchou¹.
¹University of Pennsylvania; Perelman School of Medicine, Philadelphia, PA; ²Eisenhower Medical Center; Internal Medicine Residency Program, Rancho Mirage, CA and ³University of Pennsylvania; Perelman School of Medicine, Philadelphia, PA.

Body: Background: Cholesterol-lowering agents including HMG CoA-Reductase inhibitors known as statins has been associated with improved outcomes in solid malignancies, including breast cancer (BC), however, the value of statin in BC treatment remains controversial. Prospective data from a randomized clinical trial (BIG-I 98) showed that cholesterol-lowering therapy improved outcomes in patients with ER-positive disease, but long-term impact of lipid-lowering agents across all BCs is not known.

Methods: We performed a single-institution, retrospective analysis examining the duration of statin use on the outcomes of 1523 women diagnosed with BC and underwent definitive surgery between 1995-2015. Chi-square tests and overall (OS) and disease-free (DFS) survival analysis using a Kaplan-Meier and Cox-Proportional Hazard (Cox-PH) model were performed using R. Patients were grouped into [N] = no statin use, [S] = short (<3yrs), [M] = medium (3-5yrs) and [L] = long (>5yrs) term statin use.

Results: Median age at diagnosis was 64.8yrs and 30.9% (470) were black. Over 28.7% of women received statins, with 17.0% initiating treatment after BC diagnosis. Race was a strong correlate (p<1.4 x 10-7) of statin therapy: 39.6% (186) of black women received statins (18.6% after BC diagnosis), while only 23.8% of non-black women received statins (10.4% after BC diagnosis). Over a median follow up of 70.2 months, 138 women died (84 of disease) and 125 had disease recurrence.

Outcomes Summary

<table>
<thead>
<tr>
<th>Statin Use</th>
<th>Never (N)</th>
<th>Short (S)</th>
<th>Medium (M)</th>
<th>Long (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (n)</td>
<td>1092</td>
<td>115</td>
<td>109</td>
<td>207</td>
</tr>
<tr>
<td>Local-regional Recurrence</td>
<td>72</td>
<td>7</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>Distant metastasis</td>
<td>98</td>
<td>18</td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td>No evidence of disease</td>
<td>944</td>
<td>88</td>
<td>89</td>
<td>183</td>
</tr>
<tr>
<td>Alive with disease</td>
<td>62</td>
<td>9</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Died of disease</td>
<td>61</td>
<td>9</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Died of other causes</td>
<td>15</td>
<td>6</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>Died of unknown causes</td>
<td>10</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

On univariate analysis, patients using statins had poorer OS (HR=1.48; p<0.026); interestingly, the longer the statin use i.e. [S] vs [M] vs [L], the better the OS (RR=1.99, 1.63 and 1.23, p_{LRT}<0.0173), suggesting that statin use may mitigate some of the excess mortality experienced by these patients over time. This effect was independent of hormone receptor status as the protective trend was observed in both ER+ and ER- tumors.

Importantly, using a Cox-PH model to adjust for age, metabolic comorbidities (diabetes, coronary artery disease and stroke), race, tumor and nodal stage, receptor status and extracapsular extension, we found that [S] and [M] duration of statin use was equivocal to [N], (AHR=1.30 and 0.88, respectively, p>0.4), while [L] use was protective (AHR=0.49, CI=0.22-0.90, p<0.02). The protective effect observed in the [L] group was even more pronounced on multivariate analysis for DFS. As compared to the [N] group, [S], [M], and [L] groups had improved AHR=0.68, 0.78, and 0.16 (CI=0.06-0.47) with p=0.38, 0.54 and <7.0x10-4, respectively.

Conclusions: In a retrospective cohort of >1500 women with BC, long-term statin use (>5yrs) was associated with improved OS.
and DFS. As the impact was more pronounced for DFS and after adjusting for metabolic comorbidities, treatment with statins may negate detrimental impact of metabolic disease on BC outcomes. Subanalysis by statin type and LDL levels are ongoing.
Large database visualization: Evaluating treatment patterns in metastatic breast cancer

Pravinkumar G Kandhare¹, Courtney P Williams¹, Arie Nakhmani¹, Mark E Burkard², Andres Azuero¹, Karina L Halilova¹, Maria Pisu¹, Smita Bhatia¹, Andres Forero¹ and Gabrielle B Rocque¹. ¹University of Alabama, Birmingham, AL and ²University of Wisconsin-Madison, Madison, WI.

Background: Large electronic data sources are increasingly available to clinicians and researchers; however, tools to effectively visualize data are lacking. Metastatic breast cancer (MBC) is an ideal disease to apply visualization techniques. Treatment of MBC is challenging to characterize due to the large number of treatment options and lack of a standard treatment approach.

Methods: Using SEER-Medicare data, we characterized antineoplastic treatment (hormonal medications, chemotherapy, HER2-targeted therapy) of 448 deceased MBC patients. Patients were characterized based on their estrogen receptor (ER), progesterone receptor (PR), and HER2 receptor status. Treatments from 2007-2013 were identified using Healthcare Common Procedure Coding System (HCPCS), Current Procedural Terminology (CPT) codes, or generic drug names. A graphic representing the composite treatment of MBC patients was created, with individual patients represented on the Y-axis and time on the X-axis. Specific treatments were annotated by a color-coded treatment bar, with the bar length corresponding to the patient-time on the specific treatment. The color-coding was based on the category of treatment with hormonal therapy, chemotherapy, HER2-targeted therapy, and other targeted therapies represented by shades of red, blue, green, and orange, respectively. Time after treatment until death was represented by a black line. Concurrent treatments received per patient were represented by stacked treatment bars. Kaplan-Meier curves were overlaid upon the treatment maps. Visualizations for subsets of patients were created using as grouping variables: receptor status, medication class (Her2-targeted treatments), and use of specific drugs (paclitaxel).

Results: Our sample included 266, 60, and 122 patients with ER+HER2-, ER+HER2+, and triple negative MBC, respectively. Women were predominantly Caucasian (73%), over 65 years of age at diagnosis (78%), and living in an urban area (88%). Patients within the receptor-based subsets had different treatment paradigms. The ER+HER2- visualization was predominantly red (hormone therapy). Blue (chemotherapy) was used intermittently throughout, rather than being observed primarily early or late in the treatment course. For triple negative MBC, multiple shades of blue (chemotherapy) were observed without a consistent sequencing. At the medication class level, HER2-targeted therapy was administered, if at all, across time with many gaps in HER2-targeted therapy for patients with ER+HER2+ MBC as evident from intermittent green color throughout the treatment period. Compared to standard Kaplan-Meier curves that only show survival since time of treatment in clinical trials, the visualization for specific drugs adds detail about (1) time patients are on the treatment of interest, (2) treatments received before, and (3) treatment received after the study medication.

Conclusions: We provide a method for effectively visualizing treatment data from large databases to enhance understanding of practice patterns. This technique has the potential to expedite learning from evolving electronic data sources by presenting large amounts of treatment information to clinicians and researchers in order to identify patterns to predict survival and optimize treatments.
2017 San Antonio Breast Cancer Symposium

Publication Number: P3-13-01

Title: Clinical efficacy of peri-operative aromatase inhibitor (AI) in determining long-term outcome in early breast cancer –the POETIC* trial (CRUK/07/015)

John FR Robertson¹, James P Morden², Mitchell Dowsett³, Ian Smith⁴ and Judith M Bliss². ¹University of Nottingham (Royal Derby Hospital), Derby, United Kingdom; ²Institute of Cancer Research, London, United Kingdom; ³Ralph Lauren Centre for Breast Cancer Research, London, United Kingdom; ⁴Royal Marsden NHS Foundation Trust, London, United Kingdom and ⁵Institute of Cancer Research, London, United Kingdom.

Body: Aim
To test the hypothesis that peri-operative aromatase inhibition improves long-term disease-related outcome in patients undergoing primary surgery for ER positive breast cancer

Background
Experimental evidence (Fisher et al, 1989) suggests peri-operative endocrine therapy may improve disease outcome in patients undergoing primary surgery for ER positive breast cancer, but this has not been tested clinically. POETIC (*Peri-Operative Endocrine Therapy for Individualised Care) is a phase III randomised controlled trial designed to test this hypothesis

Patients and methods
Postmenopausal patients with ER positive breast cancer were randomised 2:1 to either, peri-operative AI (centre choice: letrozole 2.5mg or anastrozole 1mg daily) for 14 days prior to and 14 days following surgery or no peri-operative treatment (Control). Randomisation was stratified by treating centre. Letrozole or anastrozole were chosen in preference to tamoxifen as i) AIs are currently the most efficacious adjuvant endocrine therapy for postmenopausal women with ER positive early breast cancer ii) AIs reach steady state after only a few days of treatment and iii) AIs have a lower risk of deep vein thrombosis. Adjuvant treatment was per UK routine practice. Tissue samples were collected at baseline and surgery (FFPE) for blinded Ki67 testing. Primary endpoint was Time to Recurrence (TTR), defined as time from randomisation to loco-regional or distant recurrence or breast cancer death with patients censored at second primary cancer or intercurrent death. Secondary endpoints include overall survival, time to distant recurrence and time to local recurrence.

Results
Between 2008 and 2014, 4480 patients (2976 AI, 1504 Control) were randomised from 130 UK centres. Median age was 67 (IQR 61-75). 18% of patients had grade 3 tumours, 39% were node positive, 61% had tumours >2cm. As of 12th June 2017, median follow-up is 60 months (IQR 48 to 71) satisfying criteria to be sufficiently mature to conduct the primary analysis. 393 TTR events have been reported. A further data snapshot, after query resolution, for the main analysis will be taken early in Q3 2017. Data to be presented include TTR by randomised treatment group, and comparison of known prognostic effects of baseline tumour characteristics on TTR in patients allocated peri-operative AI.

Discussion
POETIC will provide the largest available evidence base to assess whether a short duration of peri-operative AI improves disease related outcome for ER positive breast cancer in post-menopausal women. The results of POETIC will have important implications for clinical practice in relation to whether peri-operative endocrine therapy should become a standard of care.
2017 San Antonio Breast Cancer Symposium

Publication Number: P3-13-02

Title: Letrozole and palbociclib versus third generation chemotherapy as neoadjuvant treatment in luminal breast cancer: Results of the UNICANCER - UCBG 1-04 - NeoPAL study

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Body: Background

Benefit of neoadjuvant chemotherapy in patients (pts) with luminal breast cancer (LBC) is limited. Palbociclib combined with endocrine treatment has shown impressive results in advanced LBC. We conducted a randomized parallel phase II study, comparing a letrozole and palbociclib combination to chemotherapy as neoadjuvant treatment in LBC.

Methods

Postmenopausal women were eligible if they had a stage II-III ER-positive HER2-negative BC, not candidate to breast conserving surgery (BCS), with either a PAM50-defined luminal B tumor, or a PAM50-defined luminal A tumor profile with proven lymph node involvement. A parallel 1:1 randomization proposed 6 courses of 3rd generation chemotherapy (chemo:FEC100, 3 courses - docetaxel 100, 3 courses, q3w), or 19 weeks (wks) of letrozole (L) 2.5 mg/day plus palbocliclib (P) 125 mg/day, 3 wks on, 1 wk off. Surgery was performed at wk 20. Primary endpoint was local Residual Cancer Burden (RCB) rate. Main secondary endpoints included safety, radiological response rates, positive and negative predictive values of PAM50 ROR (risk of recurrence)-defined status in both arms, centrally reviewed RCB rates, final Ki67 value, PEPI score value and BCS rates. An interim analysis was planned when 30 patients were available for local RCB evaluation in the experimental LP arm. The protocol planned that if 5 or less than 5 local RCB 0-I (16.7%) were observed inclusions should then be stopped for futility.

Expected results

Out of 184 screened pts, 106 with Stage II-IIIA, PAM50 confirmed LBC were randomized. Non randomized patients were either Lum A N0 or non luminal per PAM50. Median PAM50 score was 70 (22-93). Patients had T1-2 (73%) or T3 (27%) tumors, and 26.5% were node positive. At interim analysis, RCB 0-I was observed in 1 and 3 pts in the LP arm and in the chemotherapy arm, respectively. The stopping boundary of 6 local RCB 0-I was not reached (3.3% - CI95% [0 ; 9.8 ]), and inclusions were then stopped as planned. Final results of the primary endpoint of the trial will be available. For both arms, local and central RCB rates will be reported, as well as safety data and all secondary endpoints.

Expected conclusions

We expect this parallel randomized phase II study to suggest that neoadjuvant LP combination leads to a low pathological complete response rate similar to chemotherapy, but also with radiological response and BCS rates alike chemotherapy with a much better safety profile. Exploratory ancillary endpoints will be reported.
**2017 San Antonio Breast Cancer Symposium**

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**Title:** KEYNOTE-173: Phase 1b multicohort study of pembrolizumab in combination with chemotherapy as neoadjuvant treatment for triple-negative breast cancer: Results from cohorts C and D

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**Body: Background:** Triple-negative breast cancer (TNBC) has limited therapeutic targets and is associated with poor outcomes. Pembrolizumab, a humanized, anti–programmed death 1 (PD-1) monoclonal antibody, has demonstrated promising antitumor activity and acceptable safety as monotherapy in the metastatic setting in both previously untreated TNBC (KEYNOTE-086 Cohort B) and previously treated TNBC (KEYNOTE-012 and KEYNOTE-086 Cohort A). KEYNOTE-173 (ClinicalTrials.gov, NCT02622074) is a 6-chort, phase 1b study of pembrolizumab combined with chemotherapy as neoadjuvant treatment in patients with locally advanced TNBC. In an initial report on Cohorts A and B in this study, pathologic complete response rates (pCR) were 60-80% at the time of definitive surgery. Here we report the results for cohorts C and D from KEYNOTE-173.

**Methods:** Between September and November of 2016, 20 patients from 8 countries were randomized to both cohorts C and D with 10 patients per cohort. Eligible patients were female, aged ≥18 years with newly diagnosed, locally advanced, nonmetastatic TNBC (stage T1c/N1-2, T2/N0-2, T3/N0-2, or T4a-c/N0-2, per American Joint Committee on Cancer (AJCC) staging criteria [7th edition]). Patients also had an Eastern Cooperative Oncology Group performance status of 0 or 1, adequate organ function, and left ventricular ejection fraction ≥50% or at least the institution lower limit of normal. Patients will receive a single dose of pembrolizumab 200 mg in cycle 1. Cycles 2-5 will include pembrolizumab 200 mg Q3W, 125 mg/m² nab-paclitaxel weekly, and carboplatin (AUC5 Q3W in C or AUC2 QW in D). Cycles 6-9 treatment will include pembrolizumab 200 mg Q3W, doxorubicin 60 mg/m² Q3W, and cyclophosphamide 600mg/m² Q3W. Therapies will be administered by intravenous infusion. Tumor biopsy will be performed at screening, after cycle 1, and after cycle 3 (optional). Breast MRI will be performed at screening, after cycle 5, and after cycle 9. Surgery will occur 3-6 weeks after treatment end. Dose-limiting toxicities (DLTs) will be assessed during cycles 1-3 and 6-7. Dose levels will be deemed toxic if ≥3 of the first 6 patients or ≥4 of 10 patients have DLTs. Primary end points are safety and recommended phase 2 dose (RP2D). Safety will be assessed in all patients who received ≥1 dose of study treatment. Adverse events will be graded per NCTCAE version 4.0. Key efficacy end points are pCR, defined as ypT0/Tis, ypN0, or ypT0 ypN0, and objective response rate (ORR) defined as the percentage of patients who achieved complete or partial response per RECIST v1.1, assessed by site radiology review. Efficacy will be assessed in the full analysis set (all patients with baseline evaluable disease [per RECIST v1.1] who received ≥1 cycle of study medication).

**Results:** Results are anticipated to be available by the time of abstract presentation.

**Expected Conclusions:** It is anticipated that pembrolizumab plus chemotherapy as neoadjuvant therapy for TNBC will result in manageable toxicity and promising antitumor activity.
Title: Glycosylation signature of HER2+ human breast cancer cell lines modulates response to trastuzumab therapy

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Body: Galectins decode glycan-containing information in various cell receptors adjusting signaling thresholds and modulating cellular functions. Upon specific binding to cell surface glycans, these lectins may induce receptor clustering, endocytosis and intracellular signaling, playing roles in many physiological and pathological conditions. In particular, galectin-1 (Gal-1), a 14.5 kDa prototype galectin, binds to terminal N-acetyllactosamine (LacNAc) residues on glycosylated proteins in the absence of α2-6 sialic acid capping. It is well documented that tumors produce Gal-1 to evade immune surveillance and promote aberrant angiogenesis mediating resistance to anti-VEGF therapy. The main goal of the present work is to explore the HER2+ breast cancer glycome and to investigate whether Gal-1 may mediate resistance to anti-HER2 targeted therapies such as Trastuzumab (TZ). We first selected three HER2+ human breast cancer cell lines with different response rate to TZ: JIMT-1, BT-474 and SK-BR-3. To identify specific glycan structures, we evaluate cell surface binding by flow cytometry with a panel of biotinylated lectins (Gal-1, LEL, SNA, PNA, PHA-L and MAA). We found that TZ-resistant JIMT-1 cells presents higher binding of Gal1 (p<0.001) and MAA (p<0.01) than the TZ-sensitive BT474 and SKBR3 cell lines, demonstrating abundant terminal LacNAc residues and higher α2-3 sialylation. In addition, BT-474 cell line exhibited higher binding of SNA (p<0.05) indicating enrichment of α2-6 sialic acid and consequent lower Gal-1 binding (p<0.001). N-glycome profiling by WAX-HPLC confirmed that JIMT-1 cell line exhibits a differential pattern characterized by abundant α2-3 and lower α2-6 sialic acid, when compared to its TZ-sensitive counterparts. This Gal-1 permissive glycophenotype was confirmed both by confocal microscopy (showing exogenous and endogenous Gal-1 binding to JIMT-1 cells) and by real-time PCR analysis of specific glycosyltransferases essential for biosynthesis of Gal-1 ligands. Furthermore, TZ-resistant JIMT-1 cells expressed higher levels of Gal-1 when compared to SK-BR-3 and BT-474 sensitive cell lines by Western blot and RT-PCR (p<0.001), further suggesting a positive autocrine loop that could contributes to tumor cell proliferation. Additionally, in order to evaluate Gal-1 implications in JIMT-1 TZ resistance, we knocked down Gal-1 in JIMT-1 derived cell line by shRNA strategies. Interestingly, knocking down Gal-1 expression sensitized JIMT-1 cells to in vitro TZ-inhibition. Moreover, treatment of BT-474 cell line with α-(2,3,6,8,9) neuraminidase A, a glycosidase capable of trimming off sialic acid from cell surface, increased cellular proliferation following Gal-1 (3 µM) exposure. Finally, in silico analysis of raw data from the Long HER Study (GSE44272) revealed that patients with poor response after TZ-treatment express higher levels of Gal-1 mRNA than long term responders, reinforcing our hypothesis from a clinical standpoint. In summary, we conclude that individual HER2+ human breast cancer cells display particular “glycosylation signatures” which, in association with the Gal-1 expression pattern, may control resistance to anti-HER2 targeted therapy and predict breast cancer clinical outcome.
Title: Chemoresistance of tamoxifen-resistant breast cancer

Qiang Liu1, Yinghua Zhu1, Yujie Liu1, Yudong Li1 and Erwei Song1. 1Sun Yat-Sen Memorial Hospital, Guangzhou, Guangdong, China.

Body: Tamoxifen resistance has been a major clinical problem and is accountable for relapse in about one third of ER positive breast cancer patients. Most of the recurrent patients will eventually receive chemotherapy. However, the chemosensitivity of these tamoxifen-resistant breast cancer patients has never been explored. In this study, we demonstrate that tamoxifen-resistant breast cancer cells express significantly more BARD1 and BRCA1, which results in the resistance to DNA-damaging chemotherapy including cisplatin and doxorubicin, but not to paclitaxel. Silencing BARD1 or BRCA1 expression or inhibition of BRCA1 phosphorylation by Dinaciclib restored the sensitivity to cisplatin in tamoxifen-resistant cells. In addition, we identified that activated PI3K/AKT pathway in tamoxifen-resistant cells was responsible for the upregulation of BARD1 and BRCA1. PI3K inhibitors, BKM120 and BYL719, decreased the expression of BARD1 and BRCA1 in tamoxifen-resistant cells and re-sensitized them to cisplatin both in vitro and in xenografted mice. Higher BARD1 and BRCA1 expression was associated with poor prognosis of early breast cancer patients, especially the ones received radiotherapy, indicating the potential use of PI3K inhibitors to reverse chemoresistance and radioresistance in ER positive breast cancer patients. This study reveals a previously unappreciated chemoresistance of tamoxifen resistant breast cancer cells, which can be overcome by PI3K inhibitors or choosing taxane-based chemotherapy. In addition to the known function of BRCA1 in triple negative breast cancer, this study indicates a novel role of BARD1/BRCA1 and PI3K pathway in the chemoresistance and radioresistance of ER-positive breast cancer. Moreover, the mechanism may not be limited to advanced breast cancer because high BARD1 expression in ER positive early breast cancer is associated with poor prognosis and resistance to radiotherapy. This study suggests that PI3K inhibitors not only help to overcome endocrine resistance, but may also reverse the resistance to DNA-damaging chemotherapy and radiotherapy in ER+ early breast cancer patients that has a 29-45% chance of PIK3CA mutation.
**Title:** CDH1 mutations predict resistance towards paclitaxel and epirubicin in primary ER positive breast cancers

Per E Lonning¹, Reham Helwa¹, Einar E Birkeland¹, Lucy R Yates², David C Wedge², Erik Lokkevik³, Hans P Eikesdal¹, Micheal R Stratton², Peter J Campbell² and Stian Knappskog¹. ¹University of Bergen, Bergen, Norway; ²Wellcome Trust Sanger Institute, Cambridge, Cambridgeshire, United Kingdom and ³Oslo University Hospital, Oslo, Norway.

**Body:** The molecular mechanisms of chemoresistance are poorly understood and predictive biomarkers guiding therapy choice are lacking.

Between January 1997 and December 2003, 223 patients with stage III or stage IV (locally advanced with limited distant metastases) were randomized to neoadjuvant treatment with either epirubicin 90mg/m²/3W or paclitaxel 200mg/m²/3W monotherapy for 4 cycles (Chrisanthar et al PLoS ONE, 2008). Responses were measured by calipers and classified according to current UICC guidelines. A total of 213 patients had evaluable responses and were included for further analyses. We applied whole genome-, whole exome- or targeted sequencing (360 gene panel; Yates et al Nat Med 2015) on snap-frozen tumor samples collected prior to therapy, in order to assess genetic alterations predicting sensitivity or resistance to chemotherapy. Next generation sequencing confirmed previous findings linking mutations in TP53 and defects in the p53 functional pathway to epirubicin resistance (Chrisanthar et al PLoS ONE, 2008). Further, mutations in the CDH1 gene (coding for E-cadherin) was associated with lack of response to epirubicin (n = 107 patients; p = 0.04) as well as to paclitaxel (n = 106; p = 0.01). This association was strong for ER+ tumors in the epirubicin (n=58; p = 0.004) as well as in the paclitaxel (n=63; p = 0.005) arm. In contrast, CDH1 mutations were less frequent among ER- tumors (only 9 harboring CDH1 mutations) and were not associated with resistance towards either epirubicn or paclitaxel. As expected, most (24 out of 34) CDH1 mutations was observed in lobular cancers (24 out of 38 lobular cancers harbored a CDH1 mutation). While lobular histology was associated with resistance to therapy in univariate analysis (p = 0.02), only CDH1 mutations remained significant in logistic multivariate analysis (p = 0.02). Further, subgroup analyses revealed CDH1 mutations to predict therapy resistance within the subgroup of lobular cancers (p=0.002).

Analyzing for defects in functional pathways, combining mutations in CDH1 and its transcriptional regulator GATA3 strengthened the association with resistance against paclitaxel (p = 0.002) as well as epirubicin (p = 0.0004) in ER+ tumors. Analyzing mRNA expression levels, we found low expression of E-cadherin to predict lack of response towards both regimens in ER+ tumours while high levels of N-cadherin was associated with lack of response in ER- tumours. Conventional EMT markers did not predict therapy resistance, and CDH1 alone had superior predictive value versus any EMT-signature.

To assess the importance of E-cadherin to epirubicin and paclitaxel sensitivity, we performed siRNA mediated knockdown in MCF-7, T47D and HCC1937 breast cancer cell lines as well as CRISPR/Cas9 mediated CDH1 knock-out in MCF-7 cells. All these in vitro experiments confirmed lack of E-cadherin function to be associated with reduced sensitivity toward both anthracyclines and taxanes across all cell lines.

In conclusion we found lack of E-cadherin function to be associated with resistance to chemotherapy both in vivo and in vitro. Breast cancers with high ER expression are known to reveal limited sensitivity toward chemotherapy in general. Our findings may potentially explain this observation.
Title: Precancerous biological risk signature for risk stratification and prevention among subjects diagnosed with atypical and non-atypical hyperplasias

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Body: Background. Patients diagnosed with proliferative hyperplasias and their clinicians face a decisional dilemma in undertaking preventive measures due to the lack of any risk stratification tools. The uncertainty surrounding the risk puts an enormous emotional burden on patients and their treating physicians and causes a dilemma in undertaking any treatments. Silbiotech in collaboration with several clinical centers has validated a biological risk signature based test that stratifies risk and guides clinicians in selecting candidates for preventive measures among atypical and non-atypical proliferative population.

Methods. A precancerous biological risk signature was first discovered using a gene expression approach. The risk signature was validated in a retrospective cohort of samples (Total, n= 508; Cases, n= 182 and Controls, n=326) from 4 clinical centers. Cancer development in Cases was after a minimum of 1 year and a maximum of 13 years. Controls were from subjects who were cancer free for a minimum of 5 years and a maximum of 20 years. The Cases and Controls had one or more of the following histologies: ADH, ALH, UDH, ULH, Papilloma, or Sclerosing Adenosis. A panel of biomarkers (MMP-1, CEACAM6, HYAL1, HEC1 and ESR1) were assayed and graded by board certified pathologists. Multiple marker data were translated into a comprehensive Cancer Risk Score using statistical approaches developed by Sibiotech, Inc. Cancer rates were assessed using Kaplan-Meier survival analysis.

Results. The Cancer Risk Score in a sample was statistically associated with cancer rates as a continuous linear variable (0-10) on a per unit basis, HR of 1.634. 95% CI [1.514, 1.764] and p= 0. The Cancer Risk Score (0-10) of a sample corresponds to risk of subsequent cancer development ranging from 2% to 85%. The cancer risk scores obtained in our cohort of samples stratified the precancerous subjects into three categories: a) Low Risk group which has a risk score of ≤ 1, b) Intermediate Risk group which has a risk score of >1 and ≤ 5 and c) Elevated Risk group which has a risk score of > 5. In the Low Risk group, cancer rate was less than 5% for at least 20 years. Among the Intermediate Risk group, cancer rate was ~ 15% in the first 5 years and increased to ~28% by 10 years. However, among the Elevated Risk group, the cancer rate was high at ~50% in the first five years after biopsy, increased to 65% by 10 years and further increased to over ~80% by 13 years.

Conclusions. Patients with proliferative precancerous growths were stratified into clinically relevant Low-, Intermediate,- and Elevated Risk groups based on the Cancer Risk Scores that were computed from the expressions of a panel of biomarkers. The cancer rates in the first five years were ~2%, 15% and 50% respectively among the 3 groups. Risk stratification by the Silbiotech test can guide clinicians in selecting the Elevated Risk group of patients for preventive measures, and sparing the Low Risk group from unnecessary treatments or mastectomies. The test validated by Silbiotech is a valuable tool in avoiding under-treatment or overtreatment of precancerous disease and preventing a significant number of cancers.
Objective: To assess whether presenting breast cancer stage has changed over time and whether there is variation with respect to race, ethnicity, and socioeconomic status.

Materials/Methods: Data from the national cancer database (NCDB) from 2004-2014 were used. We included females with invasive breast cancer and complete information on stage, race, ethnicity, insurance and income. To identify any trends over time, stage at presentation was analyzed in the whole cohort as well as by time period to assess temporal trends. Years were grouped as 2004-2006, 2007-2009, 2010-2012, and 2013-2014. Multiple logistic regression models were used to assess association between presenting stage and categorical variables using SPSS.

Results: A total of 533,972 patients met inclusion criteria. Among these women, 95.4% presented with Stage I/II disease, 4.2% with Stage II disease, and 0.4% with metastatic disease. Overall, 8.4% of women were black and 4.3% were Hispanic. 43.4% came from areas with rates of high school education (HSE) of > 29%, while 13% came from the most poorly educated districts with HSE rates of <14%. Compared to whites, black patients had a higher odds of presenting with Stage III/IV disease compared to Stage I/II (OR 1.49, 95% CI 1.43-1.55), as did Hispanic patients (OR 1.46, 95% CI 1.38-1.54) compared to non-Hispanics, and those with HSE rates of < 14% vs >29% (OR 1.42, CI 1.35-1.49).

When trends were examined by year, disparities in presenting stage of disease persisted in these groups over time, but the OR for presentation with Stage III/IV disease compared to Stage I/II disease decreased from 2004-2006 to 2013-2014 as follows: black women from 1.63 to 1.48, Hispanic women from 1.57 to 1.31, and patients with <14% rates of HSE from 1.69 to 1.35.

Women diagnosed between 2004-2006 were also more likely overall to present with Stage III/IV disease (OR 2.00, CI 1.92-2.09) compared to those patients diagnosed in 2013-2014.

Conclusions: This analysis suggests persistent disparities in presenting stage of breast cancer by race, ethnicity, and socioeconomic factors over a 10-year period, with minority populations more likely to present with Stage III/IV disease compared to more localized Stage I/II disease. It appears that gaps have narrowed over time in certain subsets including between black and white women and between Hispanic and non-Hispanic women. Despite these improvements, persistent disparities between groups point to the need for increased attention into closing these gaps.
Title: Patient navigation services in community and hospital settings provided by Avon breast cancer crusade's breast health programs

Kathryn Gates-Ferris¹, Tegan A Culler¹, Lindsay Senter¹, Carolyn Ricci² and Dana Huber². ¹CAI Global, New York, NY and ²Avon Breast Cancer Crusade, New York, NY.

Body: Background
Patient navigation (PN) has been shown to improve mammography access and breast cancer treatment among underserved women. Yet few studies have defined core data elements based on the barriers that navigators help women surmount. This study describes PN services provided by two breast health programs operated through the Avon Breast Cancer Crusade. The Avon Breast Health Outreach Program (BHOP) supports community-based organizations to conduct education and outreach to low-income and uninsured women, linking them to breast cancer screening and care. The Avon Safety Net (SN) Program funds hospital systems to navigate medically underserved women through diagnosis and treatment.

Methods
This study examined qualitative and quantitative reports of PN services documented by BHOP (n=56) and SN grantees (n=50) in 2016. Reports included six data elements describing services to address known barriers (language translation; financial assistance; transportation assistance; referral to other medical services; behavioral health referral; material support), and five write-in spaces for “other.”

Results
In 2016, BHOP programs provided over 89,500 PN services (1.7 services/client). SN programs provided over 82,800 PN services (2.3 services/client).
Appointment scheduling was the most frequently reported activity for BHOPs (57.7%), followed by translation, (14.1%), referral to financial assistance (7.1%), transportation (5.5%), and insurance enrollment (5.3%). SN programs most frequently provided translation (37.5%), followed by transportation (12%), navigation to additional medical services (11.4%), referral to financial assistance (9.5%), and care coordination (6.8%).

Discussion
The range of PN services offered by each program reflects its clients' specific point on the care continuum (i.e., screening versus diagnosis/treatment). BHOPs provide support that facilitates entry into care. In addition, BHOPs reported, in low numbers, a set of PN activities to address ongoing barriers, e.g., child care and assistance with housing, utilities, domestic violence, and immigration. SN programs, which work to reduce barriers for clients already in the health system, navigate clients to and through clinical health services.

While the present data do not show an association between PN and breast cancer outcomes, this study nonetheless demonstrates the importance of having patient navigators in both community settings and hospitals and along the entire continuum of care to facilitate women's access to breast health services. Furthermore, this study shows the utility of implementing data systems that document PN services to identify trends in known and emerging barriers to breast cancer care and ensure services are provided to address them.
Title: The influence of vitamin D (Vit D) on mammographic density (MD) and insulin like growth factor 1 (IGF1): Results from CALGB (Alliance) 70806


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Body: Rationale: There is compelling evidence that vit D may have breast cancer prevention properties. The safety and tolerability of this agent makes it a perfect candidate for chemoprevention. Chemoprevention studies focused on cancer development as the primary outcome are long and expensive. Gaining information about the feasibility of an agent using biomarker endpoints can save money and ensure that only the most promising agents move forward. This study was done to evaluate the effect of one year of vit D supplementation on several breast cancer biomarkers. Here we present the effect of supplementation on MD and serum IGF1.

Methods: CALGB 70806 is a randomized, placebo controlled phase II trial of Vit D in premenopausal women. Women were randomly assigned to either 2000IU of Vit D or placebo for 12 months, stratified by baseline (BL) Vit D level (sufficient vs insufficient [< 30 ng/mL]). Women were eligible if they were premenopausal, age <55 and had at least 25% dense breast tissue. Women were excluded if they had prior cancer, breast implants or breast reduction surgery, parathyroid disease or were taking hormone replacement, other chemoprevention agents or >400 IU/day Vit D. The study was approved by each institution’s IRB and all participants signed informed consent. Biomarker specimens were collected prior to initiation and at completion of study medication. MD was determined using the Breast Imaging Reporting and Data System (BIRADS), semi-automated and automated methods. Serum IGF1 was determined by ELISA assay. All biomarker assessments were performed at study completion.

Results: 300 women were recruited from 41 institutions across the US between 1/11-12/13. The mean age was 42.6 years and ethnicity was diverse (14% Hispanic, 12% African American). BL Vit D levels for all participants ranged from 4 - 72 ng/mL (mean 35.5 ng/mL), with 62% of participants being Vit D deficient at enrollment. 47% of women had MD between 25-50% and 12% over 50% dense. 216 (72%) women completed treatment, 8 withdrew due to side effects, and 47 for other reasons (18% withdraw rate). Women taking Vit D experienced a significant increase in Vit D levels with 99% having sufficient Vit D levels after 1 year compared to 72% in the placebo arm (P<0.0001). MD decreased 2.4% over 1 year for the entire cohort with no significant difference between arms (Table 1). Similarly, no significant change in IGF1 levels was seen with Vit D.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Placebo</th>
<th>Vit D</th>
<th>P-value</th>
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<tbody>
<tr>
<td>25(OH)D (ng/mL)</td>
<td>+0.9 (12.5)</td>
<td>+14.3 (12.9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IGF1 (ng/mL)</td>
<td>-0.1 (0.6)</td>
<td>+0.1 (0.6)</td>
<td>0.3699</td>
</tr>
<tr>
<td>MD</td>
<td>-2.4% (8.1%)</td>
<td>-2.3% (10.1%)</td>
<td>0.8953</td>
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Conclusion: Vit D supplementation with 2000 IU/day resulted in a significant increase in Vit D levels (from a mean of 35.5 to 49.7 ng/mL, p=<0.0001 and 99% with sufficient levels). Despite preliminary evidence to the contrary, no significant change in MD was observed with Vit D supplementation. This may be due to small change in MD seen over one year (and longer exposure is
needed), that only 12% of women had density >50% (a group expected to see the greatest change) or that Vit D works by another mechanism. Further study with a larger population and longer Vit D exposure is warranted.
Estrogen receptor positive luminal progenitors are decreased in the parous mammary gland

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Body: Parity (childbearing) decreases a woman's risk of developing estrogen receptor (ER) positive breast cancer. Although postulated to relate to a parity-induced reduction of mammary stem cells (MaSCs), data from previous studies are conflicting with some showing a reduction and others observing no change. We therefore compared the ability of specific mammary cell populations from parous and age-matched nulliparous mice to repopulate mammary epithelium in in vivo reconstitution assays. Repopulating activity was reduced by parity in unfractionated cells but surprisingly classical MaSC-enriched (CD24midCD49fhi) sub-populations were unaffected. By contrast, rare repopulating cells in epithelial populations distinct from MaSCs (non-CD49fhi cells) were functionally reduced by parity. Multi-parameter surface labelling showed that parity specifically reduced Sca-1+/CD49b+ ER+ luminal progenitors (LPs) and their mature Sca-1+/CD49b- ER+ derivatives. ER+ LPs were also decreased when mice were treated with the ER antagonist tamoxifen, an agent that reduces ER+ breast cancer risk. Our findings demonstrate that ER+ LPs are a target of parity-induced cellular changes in the mammary epithelium and may contribute to the parity-associated reduction in ER+ breast cancers. Given the demographic trends towards delayed pregnancy, our findings defining the cellular populations in mammary tissue most affected by parity provide specific targets to reduce breast cancer risk.
Title: Randomized double-blind placebo-controlled biomarker modulation study of vitamin D in premenopausal women at high risk for breast cancer (SWOG S0812)

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Body: Background: Several observational studies have reported an inverse association between vitamin D status and breast cancer risk. We examined whether high-dose vitamin D supplementation among premenopausal women at high-risk for breast cancer reduces mammographic density (MD), a strong predictor of breast cancer risk.

Methods: We conducted a SWOG multicenter randomized double-blind placebo-controlled trial among premenopausal women, age 18-50 years, at high risk for breast cancer (based upon a 5-year invasive breast cancer risk according to the Gail model of ≥1.67%, atypical hyperplasia/lobular carcinoma in situ, prior stage 0-II breast cancer, hereditary breast cancer syndrome, or high MD [heterogeneously/extremely dense]) and with a low baseline serum 25-hydroxyvitamin D [25(OH)D] ≤32 ng/mL. Subjects were randomized 1:1 to 12 months of standard-dose vitamin D3 600 IU daily plus vitamin D3 20,000 IU weekly or matching placebo and stratified by baseline serum 25(OH)D (<20 vs. 20-32 ng/mL) and baseline MD (≤50% vs. >50%). The primary endpoint was change in MD from baseline to 12 months, as assessed by the Cumulus technique. Secondary outcome measures included blood biomarkers (25(OH)D, 1,25(OH)D, parathyroid hormone, insulin-like growth factor [IGF]-1 and IGF binding protein-3) at baseline, 6 and 12 months, as well as change in MD at 24 months. The study has 90% power to detect a 2% absolute difference in MD between the intervention and control arms at 12 months with a standard deviation of 4%.

Results: There were 208 women enrolled from November 2011 to August 2014. Among the 201 eligible participants, median age was 44.7 years (range, 21-50); 85% were white and 92% were non-Hispanic; 32% had a baseline serum 25(OH)D <20 ng/mL and 79% had a baseline MD >50%. Among 198 evaluable participants, only grade 1-2 adverse events were reported, including 11 grade 2 mainly gastrointestinal side effects. Results from the 12- and 24-month MD and blood biomarkers will be presented.

Conclusions: This study is the first multicenter trial to investigate the biomarker effects of high-dose vitamin D supplementation in high-risk premenopausal women. A large unmet need is developing safe and effective breast cancer chemopreventive agents for premenopausal women. Results from this study will inform future vitamin D intervention trials for breast cancer chemoprevention.
Title: Presurgical weight loss trial among women with breast cancer shows little effect on tumor proliferation (Ki67) rate

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Body: Obesity is associated with higher rates of breast cancer recurrence. To explore whether weight loss (WL) has favorable effects on clinical outcomes and tumor biology, we undertook a 2-arm randomized presurgical feasibility trial among 32 overweight and obese women with biopsy-confirmed breast cancer who were scheduled for surgery. Half were assigned to an attention control group that received guidance to correct dietary deficiencies and instruction on upper arm strength training, and half received the same intervention, plus diet counseling to achieve a weight loss of 0.68–0.92 kg/week through a healthful calorically-restricted diet (energy deficit of 500-1,000 kilocalories/day) and aerobic exercise (energy deficit of 250-500 kilocalories/day) pre-surgery. Changes in weight, body composition (via DXA), diet, physical activity, quality-of-life (FACT-B), and tumor markers were assessed over the presurgical study period that averaged 30 days. The trial met all feasibility benchmarks: accrual (80%), retention (100%), and safety (absence of serious adverse events), and was powered to detect significant differences in weight change between the two study arms which also was achieved. The WL arm experienced a mean (SD) weight loss of 3.62 (1.21) kg compared to 0.52 (1.21) kg among controls (p<0.0001). Significant differences between the WL and control arms also were observed in mean (SD) baseline-to-follow-up changes in % body fat = -1.2 (0.8) vs. +0.1 (0.8) (p<0.0001) and treadmill heart rate (outcome of fitness) = -6.6 (8.6) vs. -1.5 (13.9) (p=0.028), as well as moderate-to-vigorous physical activity; median (interquartile ranges) = +189 (105-290) vs. 0 (0-60) minutes/week (p=0.0002). There did not appear to be any changes in overall QOL with this brief pre-surgical intervention; likewise, tumor proliferation (Ki67) rates showed similar mean (SD) decreases from biopsy to surgery in both arms, i.e., -4.77 (19.23) vs. -6.70 (14.49) (p=0.799). While these analyses are preliminary and we await results of tumor gene expression studies, the findings of this presurgical trial suggest weight loss may not decrease tumor proliferation rate as observed in preclinical models.
The BREAST-Q: Introducing the novel computer adaptive testing version

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Body: Background
BREAST-Q is a widely used patient-reported outcome (PRO) instrument, which measures health-related quality of life and patient satisfaction in cosmetic and reconstructive breast surgery. BREAST-Q Satisfaction with Breasts' scale has become part of the ICHOM Standard Set for Breast cancer, and is part of large national initiatives in The Netherlands, United Kingdom and United States. Shorter and more individualized assessment reduces potential respondent burden, and may further increase uptake of BREAST-Q. Computer adaptive testing (CAT) is an innovative solution to individualize and shorten scales, whilst maintaining reliability of measurement. The aim of this study was to develop a CAT version of BREAST-Q 'Satisfaction with Breasts' scale.

Methods
Item threshold properties were used to create a CAT for the 16-item BREAST-Q 'Satisfaction with Breasts' scale of the reconstruction module. During CAT simulations, each question was dynamically selected from the pool of 16 questions until a stopping rule was met. CAT administration continued until pre-specified levels of reliability were reached (standard errors (SE) of 0.32, 0.45 and 0.55). In a second sample, CAT simulations were run using responses to the 16-item scale by 5000 actual patients who had previously undergone breast reconstructions, as if these responses had been collected adaptively.

Results
Rasch Measurement Theory analysis confirmed the suitability of BREAST-Q for CAT. By applying CAT, the 'Satisfaction with Breasts' scale could be reduced to an average of 10 questions when the highest level of reliability (SE=0.32) was chosen, compared to 4 questions with the lowest acceptable reliability (SE=0.55). Score estimates were highly correlated between the CAT assessment and the full scale (correlation 0.91-0.98 in simulation 1, and 0.89-0.98 in simulation 2 using actual patient data).

Conclusion
Applying CAT to the BREAST-Q 'Satisfaction with Breasts' scale facilitates reliable assessment with 38% to 75% fewer question than the full version. BREAST-Q CAT may decrease patient burden and ease implementation in clinical practice. Results from this study are being used to further optimize BREAST-Q CAT, after which it will be made available for general use.
Title: AZD5363 in combination with fulvestrant in AKT1-mutant ER-positive metastatic breast cancer

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Body: Background: The AKT kinases are key downstream effectors of the PI3K pathway, infrequently activated directly by gain-of-function mutations. E17K is the most common activating AKT1 mutation and has recently been credentialed as an oncogenic driver and shown to be a therapeutic target in a Phase 1 basket study of AZD5363, an oral and selective pan-AKT kinase inhibitor, in patients with AKT1-mutant advanced solid tumors (NCT01226316). In heavily pretreated (median 7 prior therapies) AKT1 E17K mutant, estrogen-receptor-positive (ER+) metastatic breast cancer (MBC) patients, AZD5363 monotherapy achieved an objective response rate of 20% and median progression-free survival (PFS) of 5.5 months (95% CI, 2.9 to 6.9 months). Both preclinical and clinical data have shown that suppression of PI3K-AKT signaling results in induction of ER-dependent transcription, potentially limiting the depth and duration of response to single-agent PI3K/AKT inhibitors. Based on these data, we undertook a proof-of-concept study to explore the hypothesis that simultaneous inhibition of AKT and ER signaling would result in enhanced and more durable antitumor efficacy in AKT1-mutant ER+ breast cancer.

Methods: In a multi-cohort dose-expansion Phase 1 study (NCT01226316), we administered oral AZD5363 400 mg twice daily, 4 days on 3 days off, and fulvestrant at the labeled 500 mg dose, to patients with AKT1-mutant advanced solid tumors (NCT01226316). In heavily pretreated (median 7 prior therapies) AKT1 E17K mutant, estrogen-receptor-positive (ER+) metastatic breast cancer (MBC) patients, AZD5363 monotherapy achieved an objective response rate of 20% and median progression-free survival (PFS) of 5.5 months (95% CI, 2.9 to 6.9 months). Both preclinical and clinical data have shown that suppression of PI3K-AKT signaling results in induction of ER-dependent transcription, potentially limiting the depth and duration of response to single-agent PI3K/AKT inhibitors. Based on these data, we undertook a proof-of-concept study to explore the hypothesis that simultaneous inhibition of AKT and ER signaling would result in enhanced and more durable antitumor efficacy in AKT1-mutant ER+ breast cancer.

Results: Safety and efficacy data will be presented on patients recruited to both (FN and FR) cohorts who received at least one dose of AZD5363. Reasons for study withdrawal before week 24 due to, eg, treatment-related adverse events, disease progression, and patient decision will be detailed. Genomic biomarker analyses of plasma and tumor are ongoing and will be presented in association with clinical outcome.

Conclusions: The conclusions section will be completed once results are available.
Title: Clinical experience with nonwire nonradioactive localization SCOUT for target axillary lymph node dissection surgery

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Body: Background/Objective:
Image-guided biopsy of abnormal lymph nodes (LN) is used to assess extent of disease prior to surgery. A biopsy clip (CLIP) marks the targeted LN. Targeted axillary dissection (TAD) for selective excision of the clipped lymph node supplements surgical sentinel lymph node (SLN) assessment of pathology, including response in patients who have had neoadjuvant chemotherapy (NACT). Preoperative localization of the clipped node via wires and radioactive seeds has been described. The SCOUT device is an FDA-cleared nonwire, nonradioactive localization alternative deployed using Mammography, Ultrasound or CT guidance for use 0 - 30 days prior to surgery.

Methods:
Between July 2015 and June 2017, two breast surgeons requested SCOUT LOC in 34 patients aged 25–84 years to aid TAD of axillary LN. Preoperative SCOUT LOC was performed via an image-guidance method (27 US, 4 MG, 3 CT) tailored to patient body habitus and CLIP LN visibility. SCOUT LOC was performed on average 6 days prior to surgery (range 1-44 days). Four (4/34) patients proceeded directly to surgery without NACT and 30/34 received NACT due to Triple Negative or Her-2 positive cancer. SCOUT LOC was used supplementary to concomitant SNL in 30 patients, one with breast implants.

Results:
Surgeons successfully excised the target CLIP and LN in 34/34 patients using SCOUT localization. There were no complications attributed to SCOUT. Eleven patients were documented to have residual LN MET (2-22 mm size); 11/11 were identified via SCOUT LOC and 9/11 via SLN. Isolated tumor cells (ITC) were identified in the LN of two additional patients; 1/2 identified via SCOUT LOC and 1/2 via completion axillary dissection.

Conclusion:
This preliminary clinical experience suggests that SCOUT can aid TAD excision of the CLIP LN MET in breast surgical patients, including patients who undergo NACT and/or those who have breast implants. Our finding that 2/11 (18%) of the CLIP LN MET did not correspond to the SLN is consistent with the Z1071 trial, where up to 20% SLN did not correspond to the LN MET at surgery.
Title: Triple negative breast cancer, African ancestry and breast cancer stem cells: Unraveling the adverse triad

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Body: Triple negative breast cancer (TNBC), the most aggressive breast cancer (BC) subtype, is higher among African American (AA) compared to White American (WA) women and several studies suggest that TNBC prevalence is increased among selected populations of African patients. The colonial-era trans-Atlantic slave trade resulted in shared ancestry between contemporary AA and Ghanaian (Gh) populations, who have the highest reported TNBC prevalence (80% of all BC cases).

Breast cancer stem cells (BCSC), shown to mediate breast tumor initiation, metastasis and resistance to conventional therapy are especially abundant in TNBC in women with African ancestry. In Ghanaians, ALDH1, a marker of BCSC is very highly expressed in both benign and malignant breast tumors, but its significance is not yet known. Also, the extent to which associations between TNBC and ALDH1 are related to East African versus West African ancestry, is not known.

Utilizing immunohistochemistry, we evaluated the expression of ER, PR, HER2/neu, and ALDH1 among WA (n=153); AA (n=76); Ethiopian (Eth)/East African (n=90) and Gh. /West African (n=286) BCs. We then created patient derived xenografts based of TNBC from GH. AA and WA TNBC tumors. We isolated the ALDH+ and the CD44+/CD24- stem cell populations as well as the bulk cells from 15 PDXs using flow cytometry. We performed RNA sequencing using the Illumina HiSeq platform on the isolated cells. The data was analyzed to identify a list of differentially expressed genes and pathways between the cell populations and the samples from different ethnicities.

Some of the top genes in the ALDH+ cells were inhibited to observe their effect on the stem cell properties.

Mean age at breast cancer diagnosis was 43; 49; 60; and 57 years for the Eth; Gh; AA; and WA patients, respectively. Frequency of TNBC was significantly higher for the AA and Gh patients (41% and 54%, respectively) compared to the WA and Eth patients (23% and 15%, respectively); p<0.001. These associations were unchanged when limited to patients age 50 years and younger (47% and 49% for AA and Gh, respectively; versus 18% and 16% for WA and Eth, respectively); p<0.001. Frequency of ALDH1 positivity was also higher for the AA and Gh tumors (32% and 36%, respectively) compared to the WA and Eth tumors (23% and 17%, respectively); p=0.007.

By RNA-seq, we found the tumors to be extremely heterogeneous. The ALDH+ cells separated out irrespective of ethnicity with 14 genes separating them from CD44+/CD24- and bulk populations. They were more stem-like and had up-regulation of genes and pathways that are involved in tumor metastasis, suggesting new potential drug targets.

MMP2 and PCDH7 genes known to be involved in breast cancer metastasis were among the three top genes. Inhibiting MMP2 expression resulted in significant reduction in the ALDH+ cell population.

The data shows a correlation between West African ancestry and an increased risk of TNBC and BCs that are enriched with BCSC. In these ethnically diverse population, ALDH positivity isolated for the tumor initiating stem cells. Also, the African population, being at a higher risk for developing TNBC might hold the key to understanding the biology of this aggressive disease.
Body: Background. In the U.S., less than 20% of all breast cancer cases occur before the age of 50. Some studies have shown that younger women have poorer survival compared to older women. Proposed reasons for this difference include a more aggressive tumor profile in younger than older women, including less favorable tumor subtypes, based on steroid hormone receptor (estrogen receptor [ER/PR]/progesterone receptor [PR] and HER2 expression). We assessed differences in risk of breast cancer mortality in younger (<50 years) vs. older (50+ years) patients and evaluated differences by tumor subtype, receiving guideline appropriate treatment, or having received care at a National Cancer Institute (NCI)-designated cancer hospital.

Methods. We used data from the population-based California Cancer Registry for Stage I-III female breast cancer cases 18 years of age and older diagnosed between 2005 and 2014 with follow-up through December 31, 2014. Tumor subtypes were classified into four categories: hormone receptor (HR) positive and HER2 negative (HR+/HER2-), HR+/HER2+, HR-/HER2+, and triple negative (HR-/HER2-). Guideline-concordant care was based on receiving treatment aligned with the National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology and the American Society of Clinical Oncology Quality Oncology Practice Initiative. Multivariate Cox proportional hazards modeling was used to generate mortality rate ratios (MRR) and 95% confidence intervals (CI) for younger vs. older patients. We conducted stratified analysis by tumor subtype, guideline appropriate treatment, and receiving care at a National Cancer Institute (NCI)-designated cancer hospital.

Results: Among 156,828 breast cancer cases, 1,974 breast cancer deaths occurred in patients <50 years of age and 5,379 in those 50+ years. Risk of dying from breast cancer was higher in older than younger patients (MRR=1.13; 95% CI, 1.07-1.19). Higher MRRs comparing older to younger patients were observed for HR+/HER2- (MRR=1.25; 95% CI, 1.14-1.37), HR+/HER2+ (MRR=1.42; 95% CI, 1.18-1.71), but not for HR-/HER2+ (MRR=0.91; 95% CI, 0.77-1.08) or triple negative tumors (MRR=1.03; 95% CI, 0.94-1.13), after adjusting for multiple patient and tumor characteristics. We observed a small difference in mortality for older vs. younger women in patients who received guideline-appropriate treatment (MRR=1.09; 95% CI, 1.03-1.16) and a larger difference for those who did not (MRR=1.34; 95% CI, 1.16-1.54). Older patients who received care at a NCI-designated cancer hospital had a lower risk of dying than younger women (MRR=0.85; 95% CI, 0.72-1.00) whereas older patients who received care in other facilities had a higher risk of dying compared to younger patients (MRR=1.18; 95% CI, 1.11-1.25).

Conclusions: Our population-based study shows that breast cancer patients younger than 50 have higher survival than those 50 years and older, contrary to what has been reported from mostly single institutional studies. The age differences in survival apply to patients with HR+ but not to those with HR- tumors.
Exposure to passive smoking during childhood and risk of breast cancer - The Norwegian women and cancer study 1991-2014

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Body: Background: While active smoking is an emerging risk factor for breast cancer, the evidence is more inconsistent for passive smoking and risk of breast cancer. The purpose of this study, was to examine the effect of passive smoking in childhood on breast cancer risk in the Norwegian Women and Cancer Study, a nationally representative prospective cohort study.

Material and Methods: We followed 40 651 never smoking women, that were aged 34-70 years at enrolment, who completed a baseline questionnaire between 1991 and 2007, through linkages to national registries through December 2014. Questionnaire data included information on lifestyle factors, including lifetime history of smoking. We used Cox proportional hazards models to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) stratified by birth cohort, while adjusting for relevant confounders.

Results: During a mean follow-up of 15 years, 1 504 (never smoking) women developed invasive breast cancer, confirmed by histology. Altogether, 25,922 (63.8%) reported to be exposed to passive smoking during childhood. The age-standardised incidence rate for breast cancer was 230.1 for never and 260.9 per 100 000 person-years for passive smokers. Compared with never smokers, the women exposed to passive smoking by their father had a 17% (HR=1.17; 95% CI 1.02-1.34) higher breast cancer risk. For those reporting only mother, both parents or unknown, we found no significantly higher breast cancer risk.

Conclusion: We found that exposure to passive smoking during childhood was associated with a higher risk of breast cancer for women who reported that the father was the active smoker.
Title: Pooled analysis of two randomized phase III trials (PlanB/SuccessC) comparing six cycles of docetaxel and cyclophosphamide to sequential anthracycline taxane chemotherapy (4xEC-4xDoc or 3xFEC-3xDoc) in patients with high risk HER2-negative early breast cancer (n=6091)

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Background: Recent studies draw different conclusions, whether the omission of anthracyclines in the adjuvant treatment of patients with HER2-negative early breast cancer may reduce toxicity without significantly compromising the efficacy of adjuvant chemotherapy (Blum et al, JCO 2017, Harbeck et al, ASCO 2017)

Methods: The prospectively randomized PlanB and Success C trials compared 6 cycles of docetaxel and cyclophosphamide (DC) with either 4 cycles of epirubicin and cyclophosphamide, followed by 4 cycles of docetaxel (EC-D, PlanB) or 3 cycles of 5-FU, epirubicin and cyclophosphamide, followed by 3 cycles of docetaxel (FEC-D, SuccessC). Disease-free, distant disease-free, and overall survival (DFS, DDFS, OS) were analyzed by univariable and multivariable Cox models in the entire pooled collective and subgroups defined by factors available in both data sets.

Results:

Overall, 5923 patients with follow-up data were available for this pooled analysis (Success C: 3642 patients, PlanB: 2281 patients), with 2979 and 2944 patients randomized to A-free and A-containing chemotherapy, respectively. After 62 months median follow-up, DFS of patients receiving A-free vs A-containing chemotherapy was quite similar in univariable analysis: hazard ratio [H.R.]=1.039, 95% confidence interval [CI] 0.88 – 1.22, p=0.64) and in multivariable analysis adjusted for (locally determined) hormone receptor status [HR], age, menopausal status, type of surgery, pT, pN, histologic grade (G), histologic type and (H.R.=1.004, 95% CI 0.85 – 1.19, p=0.96). In univariable analysis, DDFS (H.R.=1.05, 95% CI 0.86 1.28, p=0.65) and OS (H.R.=0.986, 95% CI 0.78 – 1.24, p=0.90) were also similar in the entire collective.

Defining biological subtypes "luminal A-like" as HR-positive, G1/2, "luminal-B-like" as HR-positive, G3, and TN (triple negative), no significant differences were seen in DFS between A-free and A-containing chemotherapy in luminal A-like (H.R.=1.063, 95% CI 0.81 – 1.39, p=0.66), luminal-B-like (H.R.=1.071, 95% CI 0.78 – 1.48, p=0.68), or TN tumors (H.R.=0.991, 95% CI 0.76 – 1.30, p=0.95). However, in patients with four or more affected lymph nodes (pN2-3), A-containing chemotherapy was associated with significantly better DFS (H.R.=0.084, 95%-CI 0.48- 0.98, p=0.04). The benefit was seen in pN2-3 patients with luminal tumors (H.R.=0.616, 95% CI 0.41 - 0.93, p=0.021). No significant difference was seen in the small group of pN2-3 patients with triple negative tumors (H.R.=1.055, 95% CI 0.50 - 2.25, p=0.89).

Conclusion:

Our results support the hypothesis that 6 cycles of DC provide sufficient efficacy compared to anthracycline containing regimen in most patients with HER2-negative early breast cancer. However, subgroup analyses indicate that high-risk patients with more than four affected lymph nodes might benefit from anthracycline-containing chemotherapy. Further pooled analysis of all available data is warranted to provide conclusive evidence and in particular to refine estimates of potential benefit in high-risk subgroups.
Title: First results of the randomized phase II neoadjuvant GeparNuevo study investigating the addition of PD-L1 antibody durvalumab to the taxane-anthracycline containing chemotherapy in triple negative breast cancer (TNBC)

Methods:
GeparNuevo randomizes patients to durvalumab (D) 1.5 g i.v. or placebo (Pl) every 4 weeks. D/Pl monotherapy (0.75 g i.v.) is given for the first 2 weeks (window part), followed by D/Pl plus nab-paclitaxel (nP) 125 mg/m² weekly for 12 weeks, followed by D/Pl plus epirubicin/cyclophosphamide (EC) q2 weeks for 4 cycles. Randomization is stratified by stromal TILs (sTILs) (low (≤10%), intermediate (11-59%), high (≥60%)).

Primary objective is to compare pCR (ypT0 ypN0) rates. Secondary objectives are pCR rates in stratified subpopulations and according to other pCR definitions; response rates; breast conservation rate; toxicity; compliance and survival.

Results:
As of 1st June 2017, a total of 106 patients with centrally confirmed TNBC were enrolled in the study. Median age is 49.0 (range 25.0-76.0), 84.5% of tumors were ductal or ductal lobular-invasive, 85.4% grade 3, 61.2% of patients were premenopausal and 38.8% postmenopausal. Overall 34.0% of the patients had low levels of sTILs, 51.5% intermediate and 14.6% high. Median Ki67 status at baseline was 49.0% (range 3.0-96.0). The results of the first biomarker analysis performed on clinical samples collected at baseline and after the window part of the study will be presented at the meeting.

Conclusions:
GeparNuevo investigates the addition of D to the neoadjuvant CT in TNBC. This first analysis will determine if D in TNBC can increase pCR.
Title: The evaluation of a novel agent plus standard neoadjuvant therapy in high-risk breast cancer: Results from the I-SPY 2 trial

I-SPY 2 TRIAL Investigators¹. ¹I SPY 2 TRIALS, San Francisco, CA.

Body: Background: I-SPY 2 is a multicenter, phase 2 trial using response-adaptive randomization within biomarker subtypes to evaluate novel agents when added to standard neoadjuvant therapy for women with high-risk stage II/III breast cancer - investigational treatment followed by doxorubicin & cyclophosphamide(AC) q2-3 wk x 4 vs. weekly paclitaxel/AC (control arm). The primary endpoint is pathologic complete response (pCR) at surgery. The goal is to identify/graduate regimens that have ≥85% Bayesian predictive probability of success (statistical significance) in a 300-patient phase 3 neoadjuvant trial defined by hormone-receptor (HR) & HER2 status & MammaPrint (MP). Regimens may also leave the trial for futility (< 10% probability of success) or following accrual of maximum sample size (10% ≤ probability of success <85%). We report the results for an experimental arm.

Methods: Women with tumors ≥2.5cm were eligible for screening. MP low/HR+ and HER2+ tumors were ineligible for randomization. Analysis was intention to treat with patients who switched to non-protocol therapy counted as non-pCRs. This investigational arm was open only to HER2- patients, and eligible for graduation in 3 of 10 pre-defined signatures: HER2-, HR+HER2- and HR-HER2-.

Results: To be reported with placeholder update by September 15.

Conclusion: The I-SPY 2 adaptive randomization study estimates the probability that investigational regimens will be successful in a phase 3 neoadjuvant trial. The value of I-SPY 2 is to give insight about the performance of an investigational agent's likelihood of achieving pCR. We will report the results of an experimental arm by September 15.
Title: Apocrine breast carcinomas have a better prognosis than invasive ductal carcinoma not otherwise specified despite high histological grade

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Body: Background: Apocrine carcinomas are a rare breast histologic subtype with secretory features that are characterized by abundant eosinophilic granular cytoplasm and prominent nuclei. This histological subtype has historically been indistinguishable clinically from invasive ductal carcinoma not otherwise specified (NOS); however, recent findings call this observation into question such that the prognostic significance of invasive apocrine carcinoma is unclear.

Methods: We constructed a tissue microarray (TMA) containing 163 triple negative (TN), 55 Her2+ and 115 hormone receptor (HR)-positive (ER+/Her2-negative) breast carcinomas that were obtained through Markey Cancer Center clinics. Tissues were immunohistochemically stained for ER, PR, AR, Her2, ITGB4, vimentin and p53. Using a unique patient-derived xenograft model that we established from a therapy-naïve apocrine-clear cell breast carcinoma, we treated tumors with doxorubicin/cyclophosphamide (Adriamycin/Cytoxan; A/C), cisplatin, vinorelbine, paclitaxel, 5-fluorouracil (5FU) or gemcitabine and compared tumor response rates to untreated control tumors.

Results: We found that 42 of 347 (12%) breast cancers in our TMA presented with prominent apocrine features, including 18 TN, 6 Her2+, 4 Her2+/ER+ and 18 HR-positive cancers. These tumors were more frequently poorly differentiated, had a higher nuclear grade (grade 3 in 31/41) and had low levels of tumor-infiltrating lymphocytes (34/42 had leukocytes associated with carcinoma cells in < 5% of tumor area). The breast cancer cells were generally vimentin negative (35/42); and expressed elevated p53 protein levels only in the poorly differentiated tumors (21/26). Furthermore, we found that patients whose tumors had prominent apocrine differentiation had a better 10-year overall survival rate (90% for apocrine vs 59% for NOS; logrank p = 0.048) despite the fact that 25% of patients experienced recurrence or distant metastases. Using our apocrine-clear cell PDX model, we found that this tumor type was exquisitely sensitive to A/C treatment and partially responsive to vinorelbine and cisplatin. However, the PDX-tumor progressed with 5FU and gemcitabine treatment similar to untreated controls, suggesting a lack of response to these drugs.

Conclusions: Patients with apocrine breast carcinoma had a better prognosis compared to those with invasive ductal carcinoma NOS. Using a PDX model of apocrine-clear cell breast carcinoma, we find that this tumor type responds exceptionally well to A/C treatment, which may explain the improved prognosis for these tumors compared to historical observations prior to the adoption of A/C plus taxane as standard of care for breast cancer. These observations suggest that patients with apocrine cancers may benefit from A/C treatment even if they present with metastases. This study also highlights the utility of PDX modeling to understand responsiveness of select patient cohorts to chemotherapy, especially given the complex nature of modern chemotherapeutic regimens. Further studies are needed to validate these findings and determine if patients with apocrine carcinoma may benefit from A/C treatment alone, which would be aided by additional PDX models of this breast cancer subtype.